IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Senn-Bilfinger et al.

Serial No.: 10/811,496

Filed: April 1, 2004, as Continuation of U. S. Patent Application 10/182,629 filed October 1, 2002 which is a 371 of PCT/EP01/03510 filed March 28, 2001

For: Pyrano[2,3-c]imidazo[1,2-a]pyridine derivatives for the treatment of gastro-intestinal disorders

Honorable Commissioner of Patents
and Trademarks
Washington, D. C. 20231

Sir:

Declaration Under Rule 132

I, Wolfgang Kromer, declare and say:

1.1. That I am a citizen of the Federal Republic of Germany, residing at Hinterhauserstr. 5, D-78464 Konstanz, Germany.

That, from 1965 to 1972, I studied Medicine at the Universities of Erlangen and Würzburg, Germany.

That I received the degree of Doctor of Medicine in 1973 and was admitted to practice on Dec. 10, 1973.

That I was Medical Assistant at Hospital from Oct 1972 to Nov. 1973 and Ward Physician (last at the Munich University Hospital, Clinic of Neurology) from Dec. 1973 to Aug. 1975, and thereafter Research Assistant at the Institute of Pharmacology at Munich University.

That I received the license as specialist in experimental pharmacology and toxicology on Dec. 16, 1980, and as specialist in clinical pharmacology on March 1, 1991.

That I received the degree as a university teacher (Dr. med. habil.) from Munich University on Nov. 19, 1981, and accepted a position as Professor of Pharmacology at Hannover Medical School on April 15, 1982.

That I had a sabbatical at the National Institute for Medical Research in London, UK, for 6 months in 1984.

That I have been appointed Head of Department of Gastrointestinal Pharmacology at Byk Gulden Lomberg Chemische Fabrik GmbH in Konstanz, Germany, on May 2, 1985, which position I still hold today.

That I am the author of currently 75 original publications and scientific reviews as well as additional congress abstracts, and lectured pharmacology for many years at the university.

That I am thoroughly familiar with evaluating chemical compounds for their antisecretory and protective action in the gastrointestinal tract of animals and humans.

That I am fully conversant with all pharmacological and structure-activity aspects of acid pump antagonists, including the chemical classes that are subject of application Serial No. 10/811,496 and U. S. Patent No. 6,160,119.

1.2. That, in order to show the unexpected and advantageous properties of the compounds of application Serial No. 10/811,496, the following comparative tests were performed in the laboratories of ALTANA Pharma AG (the former Byk Gulden Lomberg Chemische Fabrik GmbH) under my supervision and direction:

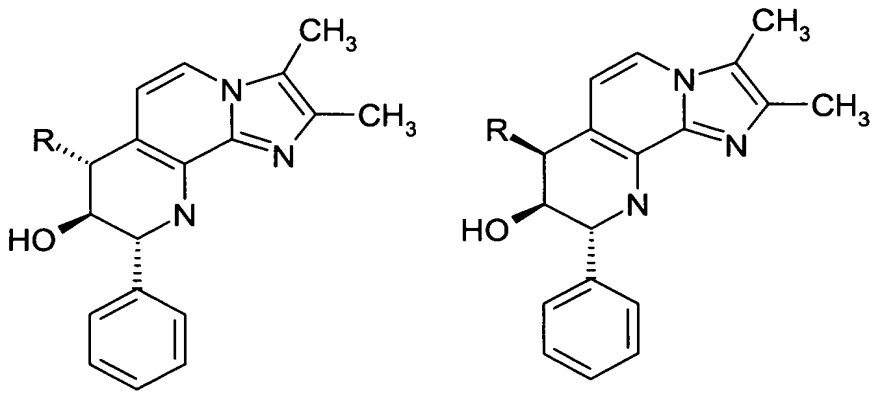
2. Comparative Tests

In order to show that the compounds of application Serial No. 10/811,496 are patentably distinct from the compounds of U. S. Patent No. 6,160,119, comparative tests have been carried out under my supervision. The compounds listed in the following ***Table 1*** have been comparatively investigated in the Ghosh-Schild rat upon intraduodenal administration (***Table 2***, for methodical details, see Kromer et al., J. Pharmacol. Exp. Ther. 254, 129-135, 1990).

2.1. Compounds

Table 1

Compounds investigated



	7,8-trans-compounds	7,8-cis-compounds
R = HO	Example 8, USP 6,160,119 * (A)	Example 8, USP 6,160,119 * (G)
R = $CH_3OC_2H_4O$	Example 1, Ser. No. 10/811,496 (B)	Example 2, Ser. No. 10/811,496 (H)
R = C_2H_5O	Example 3, Ser. No. 10/811,496 (C)	Example 4, Ser. No. 10/811,496 (I)
R = $CH_3OC_3H_6O$	Example 5, Ser. No. 10/811,496 (D)	Example 6, Ser. No. 10/811,496 (J)
R = $(CH_3)_2CHO$	Example 7, Ser. No. 10/811,496 (E)	Example 8, Ser. No. 10/811,496 (K)
R = C_4H_9O	Example 9, Ser. No. 10/811,496 (F)	Example 10, Ser. No. 10/811,496 (L)

* Example 8 of USP 6,160,119 describes the racemic mixture of the 7,8-trans- and the 7,8-cis-dihydroxy compounds. The pure diastereomers have been synthesized and isolated in order to have for each individual compound of Ser. No. 10/811,496 the structurally closest related prior art counterpart. The alphabetic characters in brackets refer to the compound identifications given in table 2.

The comparative tests were carried out in order to show that the trans-compounds ("trans" with regard to the groups R and OH in positions 7 and 8) of Ser. No. 10/811,496 are patentably distinct from their structurally closest related counterpart in USP 6,160,119, namely the trans-diol of Example 8 in USP 6,160,119, and that the cis-compounds of Ser. No. 10/811,496 are patentably distinct from their structurally closest related counterpart in USP 6,160,119, namely the cis-diol of Example 8 in USP 6,160,119.

2.1. Methods

2.1.1. Ghosh-Schild rat (GSR)

These experiments were done according to Ghosh and Schild (Br. J. Pharmacol. 13, 54-61, 1958). Female Sprague-Dawley rats, 170-240 g body weight, deprived of food for 24 hours before the experiment with free access to water, were anesthetized with urethane and tracheotomized. After a midline abdominal incision, a PVC tube was inserted into the stomach via the esophagus and the stomach was perfused with saline (37 ^{0}C) at a rate of 0.5 ml/min. A second tube draining the pylorus was inserted through the abdominal wall for collection of gastric secretion. Acid secretion was determined at 15 min intervals by titration of the perfusate with 0.01 N

NaOH to pH 7. Gastric secretion was stimulated during a period of 4.5 h by an intravenous infusion of pentagastrin (1 µg/kg/min) starting 30 min after determination of two basal values of acid secretion. Test drugs were administered intraduodenally 60 min after the start of pentagastrin infusion. The dose-response relationship was established using the maximum and mean (3.5 h) %-inhibitions of acid secretion by the particular dose, compared to the pre-drug value of acid output.

2.1.2. Statistics

In the acid output studies, means with 95% confidence limits have been used. The doses which caused 50% inhibition (ED_{50} values with 95% confidence limits) were interpolated from dose-response curves according to Metzler et al. (for ref., see Kromer et al., Pharmacology 60, 179-187, 2000) and Unkelbach and Wolf (for ref., see Kromer et al., Pharmacology 60, 179-187, 2000).

2.2. Results

2.2.1. Ghosh-Schild rat (GSR)

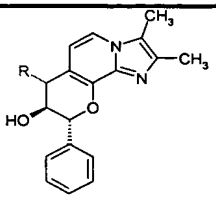
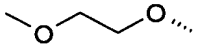
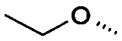
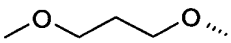
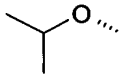
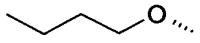
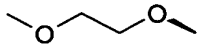
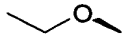
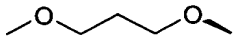
Table 2 shows two groups of comparative test data, a first group (left column of table 1) where the trans-diol-compound of USP 6,160,119 (the trans-diol of Example 8 = compound A in table 2) was compared with its trans-methoxy-ethoxy, trans-ethoxy, trans-methoxy-propoxy, trans-isopropoxy and trans-butoxy counterparts of Ser. No. 10/811,496 (Examples 1, 3, 5, 7 and 9 = compounds B-F in table 2), and a second group (right column of table 1) where the cis-diol of USP 6,160,119 (the cis-diol of Example 8 = compound G in table 2) was compared with its cis-methoxy-ethoxy, cis-ethoxy, cis-methoxy-propoxy, cis-isopropoxy and cis-butoxy counterparts of Ser. No. 10/811,496 (Examples 2, 4, 6, 8 and 10 = compounds H-L in table 2), respectively.

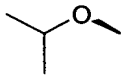
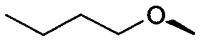
The data of both groups clearly demonstrate - with the exception of Example 6 of Ser. No. 10/811,496 (Compound J in Table 2) - an unexpected increase in potency of the compounds of Ser. No. 10/811,496 as compared with the respective compounds of USP 6,160,119 (i.e., compounds B-F versus compound A, and compounds H-L versus compound G).

Table 2

LUMENPERFUSED RAT STOMACH IN VIVO (GHOSH-SCHILD RAT)

Pentagastrin stimulation: 1 µg/kg.min i.v.

Compound		Inhibition of stimulated acid output following **intraduodenal administration**							
				Maximum			Mean 3.5 h		
		N	Dose µmol/kg i.d.	%	p	ED50 µmol/kg	%	p	ED50 µmol/kg
A		5	0.6	33	*	1.03	24	*	1.71
		5	1.0	37	*	(0.81 - 1.30)	27	*	(1.34 - 2.17)
		5	2.0	82	*		48	*	
		5	3.0	104	*		78	*	
B		5	0.2	25	*	0.25	13	*	0.36
		5	0.3	72	*	(0.23 - 0.27)	45	*	(0.33 - 0.40)
		5	0.6	102	*		75	*	
C		5	0.1	37	*	0.16	28	*	0.27
		5	0.2	52	*	(0.12 - 0.20)	35	*	(0.20 - 0.35)
		5	0.3	78	*		55	*	
		4	0.6	96			75		
D		5	0.2	18	*	0.50	7	ns	0.84
		5	0.3	31	*	(0.39 - 0.65)	20	*	(0.64 - 1.10)
		6	0.6	50	*		33	*	
		4	1.0	80			60		
		3	2.0	108			81		
E		5	0.2	21	ns	0.29	12	ns	0.40
		4	0.3	55		(0.25 - 0.33)	42		(0.34 - 0.47)
		5	0.6	89	*		68	*	
		3	1.0	112			90		
F		5	0.2	16	ns	0.39	4	ns	0.65
		5	0.3	50	*	(0.28 - 0.54)	38	*	(0.46 - 0.92)
		5	0.6	59	*		37	*	
		4	1.0	83			62		
		3	2.0	123			101		
G		6	0.6	28	*	1.26	20	ns	2.01
		5	1.0	38	*	(0.98 - 1.63)	26	*	(1.55 - 2.60)
		5	2.0	62	*		44	*	
		5	3.0	90	*		69	*	
		3	6.0	104			79		
H		5	0.3	26	*	0.59	16	*	0.98
		4	0.6	55		(0.45 - 0.77)	30		(0.82 - 1.17)
		4	1.0	59			44		
		3	2.0	102			85		
I		5	0.1	27	*	0.21	16	ns	0.34
		5	0.2	43	*	(0.17 - 0.25)	31	*	(0.27 - 0.42)
		5	0.3	63	*		43	*	
		4	0.6	90			70		
		3	1.0	106			91		
J		6	2.0	23	*	2.87	15	ns	3.45
		5	3.0	50	*	(2.50 - 3.29)	41	*	(2.94 - 4.05)
		3	6.0	109			83		

Compound		Inhibition of stimulated acid output following **intraduodenal administration**							
				Maximum			Mean 3.5 h		
		N	Dose µmol/kg i.d.	%	p	ED50 µmol/kg	%	p	ED50 µmol/kg
K		5	0.2	32	ns	0.36	21	ns	0.53
		5	0.3	39	*	(0.27 - 0.48)	24	*	(0.41 - 0.68)
		4	0.6	60			45		
		3	1.0	107			91		
L		5	0.3	25	ns	0.57	12	ns	0.99
		5	0.6	56	*	(0.44 - 0.74)	38	*	(0.73 - 1.34)
		5	1.0	62	*		40	*	
		3	2.0	117			86		

* = significant ($p < 0.05$); ns = not significant ($p \geq 0.05$); () = 95% confidence limits.

3. Discussion

The data compiled in Table 2 show beyond any doubt that the compounds claimed in Ser. No. 10/811,496 display in the comparative tests an unexpected progress in the dose (µmol/kg)-related antisecretory potency, as compared with their structurally closest related counterparts of USP 6,160,119.

Table 2 shows that - with exception of Compound J - both in the trans- and the cis-series, the methoxy-ethoxy, ethoxy, methoxy-propoxy, isopropoxy and butoxy counterparts of Ser. No. 10/811,496 derivatives achieved 50%-inhibitions of gastric acid secretion (ED50) at considerably lower doses than their structurally closest related counterparts of USP 6,160,119.

Based on our experience, it is reasonable to assume that compounds effective in the GSR are also effective in man.

4. The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon.

Signed at Constance,
Federal Republic of Germany,

this 06 day of July, 2004.

.....

Prof. Dr. med. Wolfgang Kromer

Original Paper

Pharmacology

Pharmacology 2000;60:179–187

Received: May 25, 1999
Accepted: September 1, 1999

Animal Pharmacology of Reversible Antagonism of the Gastric Acid Pump, Compared to Standard Antisecretory Principles

Wolfgang Kromer Stefan Postius Richard Riedel

Department of Pharmacology, Byk Gulden, Constance, Germany

Key Words

Acid pump antagonists · Pumaprazole · Animal experiments · Acid output · Intragastric pH metry · Ranitidine · Pirenzepine · Omeprazole

Abstract

To define the basic antisecretory profile of a potassium-competitive antagonism of the gastric acid pump relative to other classes of acid-inhibitory drugs, they were compared to each other against all three major stimuli of acid secretion. Pumaprazole is an imidazo-pyridine derivative that was used in this investigation as an example of reversibly binding acid pump antagonists (APAs). It differs from covalently binding proton pump inhibitors (PPIs), such as omeprazole, both with respect to chemical structure and mode of interaction with the gastric H^+/K^+-ATPase (i.e., the acid or proton pump). The present data show that a single dose of pumaprazole is able to elevate intragastric pH in the dog with gastric fistula under pentagastrin or carbachol stimulation from pH 1 to about pH 7 while still displaying a dose-dependent, well-controlable duration of action of a few hours. Ranitidine at the same oral dose also shows a short duration of action, but combined with a far lower efficacy. By contrast, a single oral dose of the PPI omeprazole elevates intragastric pH for a longer time period, but this pH elevation is far lower compared to that of the APA. Regarding the less stringent parameter of inhibition of total acid output in the Heidenhain pouch dog, the modified Shay rat or the Ghosh-Schild rat, pumaprazole is, overall, slightly more efficacious than ranitidine. The M_1-muscarinic antagonist pirenzepine is ineffective (against histamine stimulation) or far less effective than pumaprazole (against pentagastrin-stimulation), but as effective as pumaprazole against carbachol stimulation in the Ghosh-Schild rat. Basal acid output in the same model is more effectively inhibited by pumaprazole than by ranitidine. In conclusion, our data demonstrate the exceptional ability of a reversibly binding APA to elevate intragastric pH up to neutrality even upon a first administration while still displaying a limited, dose-dependent duration of action.

Introduction

Drugs capable of potently suppressing gastric acid secretion provide a mainstay in the therapy of acid-related diseases [1–4]. This still holds true after the identification of *Helicobacter pylori* as the major pathogen in gastric and duodenal ulcer disease [5, 6]. The main stimulus for gastric acid secretion in man is histamine [7, 8], and therefore H_2-receptor antagonists constitute a well recog-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0031–7012/00/0604–0179$17.50/0

Accessible online at:
www.karger.com/journals/pha

Prof. Wolfgang Kromer, MD
Department of Pharmacology, Byk Gulden
Byk-Gulden-Strasse 2
D–78467 Konstanz (Germany)
Tel. +49 7531 84 2628, Fax +49 7531 84 9 2628, E-Mail Wolfgang.Kromer@byk.de

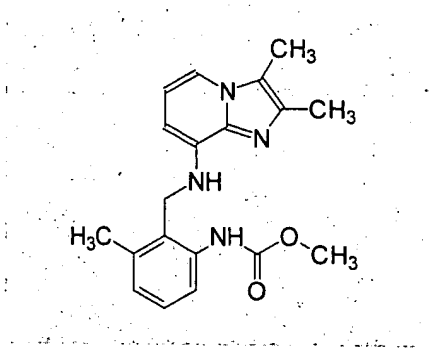


Fig. 1. Chemical structure of pumaprazole (B9208-041), free base.

nized therapeutic principle. However, some degree of tolerance develops to H_2-receptor antagonists which may be clinically relevant in a certain number of patients [9–11], and 5–10% of duodenal ulcer patients turn out to be resistant to H_2-receptor antagonists [12]. Proton pump inhibitors (PPIs) are substituted benzimidazoles that block the gastric proton pump by covalent binding [13–16] and thus overcome this therapeutic problem. Because they need to be activated inside the acidic canaliculus of secreting parietal cells, their full pH-elevating effect in the stomach only develops over 2–3 days. Actually, parietal cells resting during drug exposure will remain unaffected so that no optimal symptom relief may be achieved in some patients after the first dose [17]. By contrast, acid pump antagonists (APAs) neither need to be activated by acid within the canaliculus of the parietal cell, nor do they covalently react with SH groups of the proton pump like PPIs [18]. Because they compete with potassium binding on the extracytoplasmic face of the catalytic (α) subunit of the acid pump [19, 20], APAs immediately interrupt the transport cycle of the acid pump; however, only for limited periods of time determined by their plasma elimination half lifes and the doses administered. Interestingly, these drugs seem to bind already to the resting pump, i.e., to its non-phosphorylated form in the absence of ATP [21].

In the present investigation, pumaprazole was used as a tool to define the pharmacologic profile of acid pump antagonism in different animal models relative to other antisecretory principles, i.e., H_2-receptor antagonism, M_1-muscarinic receptor antagonism, and covalent proton pump blockade. Pumaprazole is 8-[(2-methoxycarbonylamino-6-methyl-phenyl)-methylamino]-2,3-dimethylimidazo[1,2-a]pyridine (MW 338.41; 1 μmol = 0.338 mg; fig. 1). The suffix '... prazole' has been selected by the WHO to address the inhibition of the acid (proton) pump as seen with substituted benzimidazoles (also named '... prazoles') although the mode of action of the two groups of drugs is completely different (competitive vs. covalent binding). Pumaprazole is a weak base with a pK_a of 7.2 thus accumulating in the acidic space of a secreting parietal cell. In contrast to PPIs, however, its target selectivity for the parietal cell acid pump is based on a specific fit between its chemical structure and its target. The IC_{50} of pumaprazole is 0.04 μmol/l in an H^+/K^+-ATPase preparation (open gastric vesicles) vs. 63 μmol/l in a sodium pump preparation from dog kidney (1 mmol/l K^+ in either case). This results in a selectivity factor of pumaprazole under these conditions of more than 1,000 in favor of the acid pump (Dr. W.A. Simon, personal commun.).

Materials and Methods

General

All animal experiments were approved by the responsible authority on the basis of the German Animal Protection Law.

Acid Secretion in the Lumen-Perfused Rat Stomach in situ (Ghosh-Schild Rat)

These experiments were done according to Ghosh and Schild [22]. Female Sprague-Dawley rats, 170–240 g body weight, deprived of food for 24 h before the experiment with free access to water, were anesthetized with urethane (1.5 g/kg i.m.) and tracheotomized. After a midline abdominal incision, a PVC tube was inserted into the stomach via the esophagus and the stomach was perfused with saline (37°C) at a rate of 0.5 ml/min. A second tube draining the pylorus was inserted through the abdominal wall for collection of gastric secretion. Acid secretion was determined at 15-min intervals by titration of the perfusate with 0.01 *N* NaOH to pH 7. Gastric secretion was stimulated during a period of 4.5–5 h by an intravenous (i.v.) infusion of either histamine 0.6 μmol × kg^{-1} × min^{-1}, pentagastrin 1 μg × kg^{-1} × min^{-1}, or carbachol 0.002 μmol × kg^{-1} × min^{-1}, starting 30 min after determination of 2 basal values of acid secretion. These doses of the secretagogues have been selected from dose-response curves and correspond to between 50 and 80% of maximum acid output (fig. 2). The selection criteria were submaximal stimulation in order to guarantee selectivity of stimulation, avoidance of unacceptable cardiovascular side effects in the animals (particularly with carbachol), and as constant (plateau) stimulation over time as possible. In order to assess the potencies (ID_{50}s) and efficacies (maximum achievable inhibitions) of the antagonists against different secretagogues independent of potential pharmacokinetic influences, the test drugs were administered by an intravenous (i.v.) bolus in a volume of 1 ml/kg, and compared to intraduodenal (i.d.) administration in 2.5 ml/kg, 60 or 90 min after commencement of stimulation (see figures). The dose-response relationship was established either using the area under the curve over 3.5 h (starting at the commencement of the plateau phase) or the maximum inhibitions of acid output by the particular dose. Maximum inhibition was defined as the

maximum difference, achieved at any defined time point, between the acid output in the presence of the test drug and in the respective controls [23, 24]. These differences have been converted to percentage acid output normalized to the respective control value (100%).

Acid Secretion and Gastric Lesions in the Modified Shay Rat

This is the only model that allows parallel assessment of acid output and lesion index in the same animal. The experimental procedure according to Shay et al. [25] and modified by Okabe et al. [26] was used. The pylorus of female Sprague-Dawley rats (160–220 g body weight, fasted for 24 h with free access to water) was ligated under ether anesthesia, the abdomen was closed and 100 mg/kg of acetylsalicylic acid (ASA) in 10 ml/kg were given orally. ASA causes both mucosal lesions and peripheral analgesia. Following ASA administration, the test substance (solution) or vehicle (i.v. saline or tap water in case of oral administration) was given i.v. in 1 ml/kg or i.d. in 2.5 ml/kg. Oral drug administration (in 10 ml/kg) was 1 h before pylorus ligation. Four hours after pylorus ligation, the stomach was excised, carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed, centrifuged, the volume was measured and the acidity determined by titration with 0.1 *N* NaOH to pH 7. The mucosa was flushed with saline and the stomach pinned on a plate. The number and size of mucosal lesions were evaluated blind as to drug treatment using a stereomicroscope with 10-fold magnification. A 6-point score was used to assess the diameter of the lesions: 0 = no ulceration; 1 = 0.1–1.4 mm; 2 = 1.5–2.4 mm; 3 = 2.5–3.4 mm; 4 = 3.5–4.4 mm; 5 = 4.5–5.4 mm, and 6 ≥ 5.4 mm. For each lesion score observed in a particular animal, the number of lesions was counted and this number multiplied by the corresponding score. The total sum of these numbers per animal represents the individual lesion index, which was compared to that of drug-free controls.

Acid Secretion in the Heidenhain Pouch Dog

Male Beagle dogs (Velaz, Prague, Czechia), aged 2–8 years, with Heidenhain pouch [27], modified after Gregory and Tracy [28] were used. Their body weight was between 15 and 20 kg. The animals were kept at a 12-hour light-dark rhythm, housed singly. They received standard food once daily at 10.00 a.m. and tap water ad libitum, and were fasted for 22 h prior to the experiment, with free access to water. Measurement of acid output was performed in 15-min fractions. After a basal period of 30 min, gastric secretion was stimulated by continuous i.v. infusion of either histamine (0.125–0.25 μmol × kg^{-1} × h^{-1}) or carbachol (6 μg × kg^{-1} × h^{-1}). This results in a 50–80% stimulation of maximum acid secretion. Oral or i.v. bolus administration of the test drug was 90 min after commencement of histamine or carbachol infusion, i.e., at time point 120 min. Stimulated acid secretion was measured from 30 to 240 min. The test drugs were administered either i.v. or orally in 0.1 ml/kg. The dose-response relationship was established based on maximum inhibitions relative to the corresponding intraindividual control values.

pH Metry in the Fasted, Gastric Fistula Dog

Male Beagle dogs (Velaz, Prague, Czechia) were chronically instrumented at an age of 1–2 years. A metallic (V4A) fistula was placed on the left side of the abdomen at the lowest part of the distal gastric corpus region near the greater curvature [29]. The animals recovered quickly and were trained for the study conditions about 3–4 weeks after the operation. The actual age of the dogs at the time of the present investigation was between 2 and 9 years, i.e. 1–8 years after operation. The animals were singly housed at 20–22 °C, 55–65% relative humidity, with a 12/12-hour light/dark cycle. They received standard food at 10 a.m. Their body weight was between 14 and 21 kg.

Continuous pH metry was started at 8 a.m. The animals were fasted for 22 h prior to the experiment with free access to water. Gas-

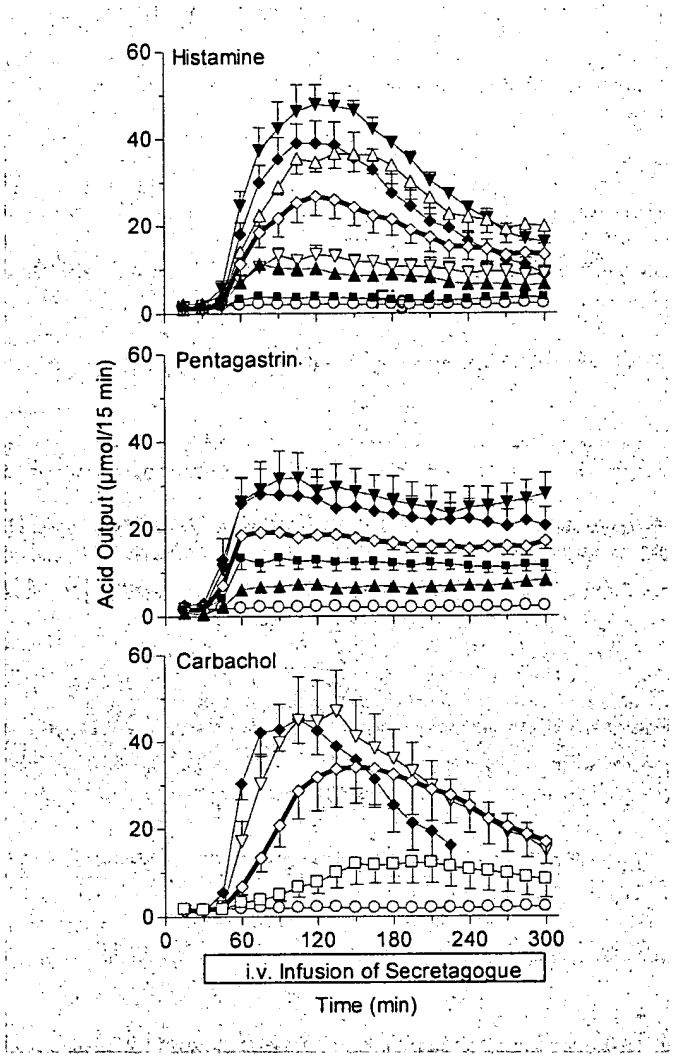


Fig. 2. Acid output (mean ± SEM) in the Ghosh-Schild rat. Dose-response curves for histamine (n = 6), pentagastrin (n = 5), and carbachol (n = 6). Secretagogues were administered as a continuous i.v. infusion, starting at 30 min. The heavy lines (◇) indicate the doses selected for the experiments shown in figures 3–5. Histamine (μmol × kg^{-1} × min^{-1}) 0.03 (■), 0.1 (▲), 0.3 (▽), 0.6 (◇), 0.8 (△), 1 (▼), 3 (◆), controls (○); pentagastrin (μg × kg^{-1} × min^{-1}) 0.1 (▲), 0.25 (■), 1 (◇), 10 (▼), 30 (◆), controls (○); carbachol (μmol × kg^{-1} × min^{-1}) 0.001 (□), 0.002 (◇), 0.003 (▽), 0.006 (◆), controls (○). At 0.006 μmol × kg^{-1} × min^{-1} carbachol, 50% of the animals died during the experiment (after time point 225 min). Note that, in figures 2–5 and 7, any failure to display a horizontal bar for variation indicates that SEM was within the size of the symbol.

tric acid secretion was stimulated, starting at noon, by subcutaneous infusion of either pentagastrin or carbachol (both 6 μg × kg^{-1} × h^{-1}). This technique was developed by Postius et al. [30] since, under basal and fed conditions, spontaneous pH fluctuations would otherwise prevent any reliable differentiation between the actions of different drugs in this model. A programmable infusion pump (Chronomat, Fresenius, Germany) was used to cover a 20-hour period. Drugs or vehicle (0.9% NaCl) were administered orally as capsules. For the time course of a single experiment, see results (fig. 8). Intragastric pH was continuously recorded by means of a combined glass electrode (Type 440 M3, Ingold, Switzerland) as suggested by McLauchlan et al. [31] and Bauerfeind et al. [32].

The electrode was connected to an ambulatory pH meter with a solid-state storage unit (Digitrapper, Synectics, Sweden). The pH-monitoring system was calibrated at 37 °C using commercial buffers of pH 1 and 7. The infusion pump and the pH-metry unit were put on the dog in specially designed frogs. After each run, recalibration at 37°C was performed to determine the drift of the electrode. A drift of less than 0.2 pH units during a 24-hour period was tolerated. Electrodes displaying a greater drift or occasional artificial pH changes were discarded. These precautions proved to be necessary since this type of electrode is extremely sensitive to mechanical distortion which may occur during a 24-hour run in freely moving animals. The electrodes were tightly fixed within the gastric fistula, protruding 10 mm into the gastric lumen. This procedure guaranteed reproducible positioning and continuous as well as sufficient soaking of the glass membrane and diaphragm of the pH electrode even during night time in the sleeping position of the animal.

At the end of each run, pH readings (5,760 readings within 24 h, each covering 15 s) were transferred to a PC using the 'Oesophogram' from Synectics (Stockholm, Sweden), yielding individual 24-hour pH profiles. Applying 'Statphac' from Synectics the individual pH profiles of 1 treatment group were processed to establish medians with 25 and 75% quartiles for intervals of 10 min each, covering a total of 24 h. The quartiles are calculated from 40 medians based on 15 s each (= 10 min).

Statistics

In the acid output studies, depending on the particular statistical distribution of the data, either means (all models except for Shay rat) or medians (Shay rat) with 95% confidence limits have been used (see fig. 6). The latter were calculated according to Hodges-Lehmann and Moses [cited in 33]. The doses which caused 50% inhibition (ID_{50} values with 95% confidence limits; fig. 6) were interpolated from dose-response curves according to Metzler et al. [23] and Unkelbach and Wolf [24]. Apart from that, descriptive presentation of the data was considered sufficient, and no further statistical treatment was performed.

Drugs

ASA, carbachol (Doryl®), and histamine-HCl were from Merck (Darmstadt, Germany); omeprazole (pellets) from Astra-Hässle (Mölndal, Sweden); pentagastrin from Sigma (St. Louis, Mo., USA), pirenzepine-dihydrochloride (prepared by the Chemical Department of Byk Gulden, Constance, Germany, for rat experiments, or purchased as ampules from Ratiopharm GmbH + Co., Germany, for dog experiments); pumaprazole from Byk Gulden and ranitidine-HCl (Zantic®) from Glaxo (Bad Oldesloe, Germany) for rat experiments and pH metry in dogs, or from Sigma for dog experiments on acid output.

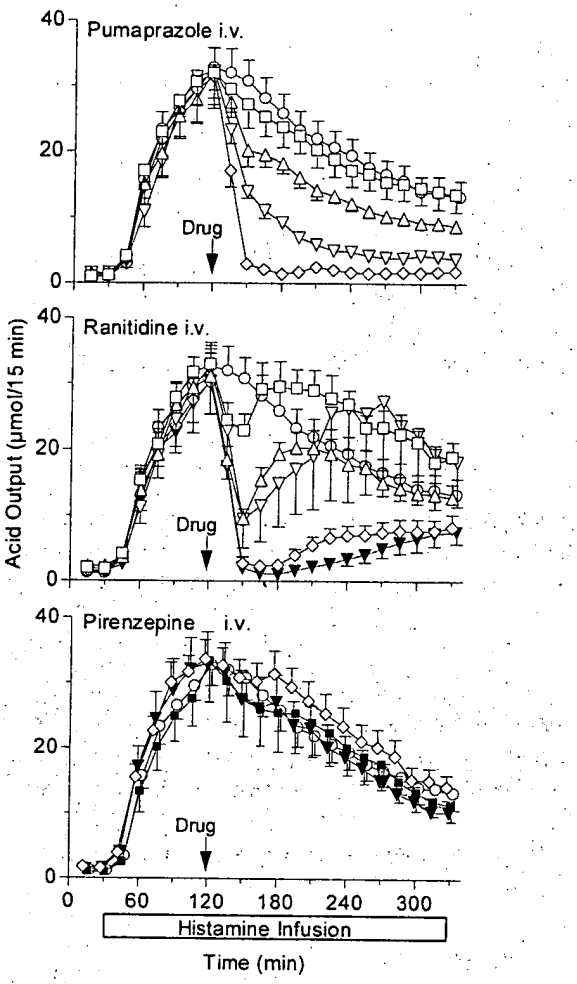


Fig. 3. Acid output (μmol/15 min; mean ± SEM) in the Ghosh-Schild rat stimulated by 0.6 μmol × kg^{-1} × min^{-1} histamine (i.v. infusion). Symbols (μmol × kg^{-1}; i.v. bolus of antisecretory drugs): 0.1 (□), 0.3 (△), 1 (▽), 3 (◇), 10 (▼), 30 (■), 100 (●), 300 (◆), controls (○). Note that symbols refer to figures 3–5, and not all doses were used in any experiment. For number of experiments, see legend to figure 6.

Results

Acid Secretion in the Lumen-Perfused Rat Stomach in situ (Ghosh-Schild Rat)

Using histamine as a stimulus, pirenzepine was completely ineffective, and ranitidine was slightly less efficacious than pumaprazole (fig. 3). When pentagastrin was used as a stimulus (fig. 4), ranitidine displayed a similar efficacy with a shorter duration of action compared to pumaprazole, while pirenzepine showed a poor inhibition

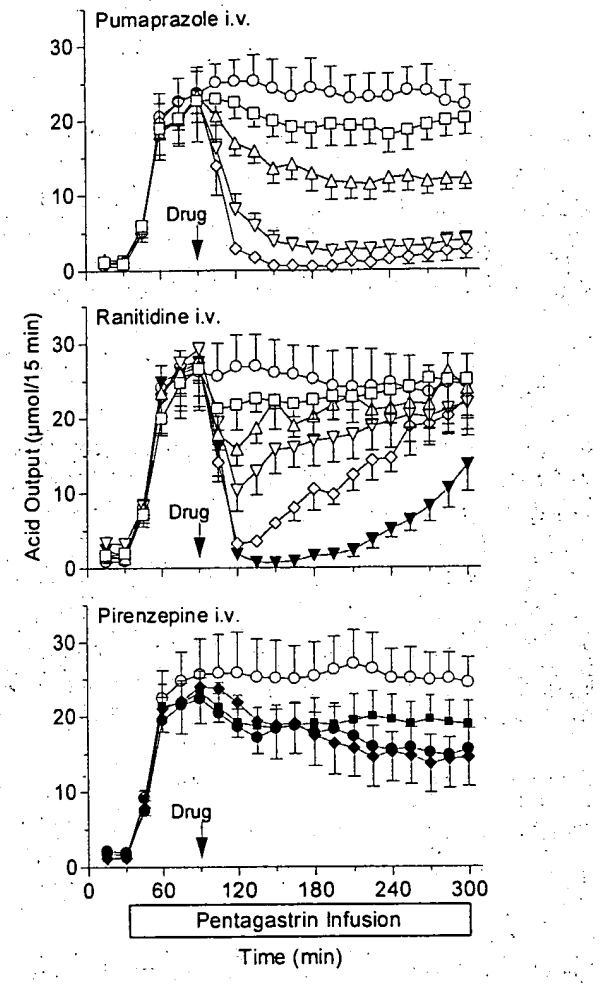


Fig. 4. Acid output (μmol/15 min; mean ± SEM) in the Ghosh-Schild rat stimulated by 1 μg × kg^{-1} × min^{-1} pentagastrin (i.v. infusion). Drug administration by i.v. bolus. For symbols see legend to figure 3.

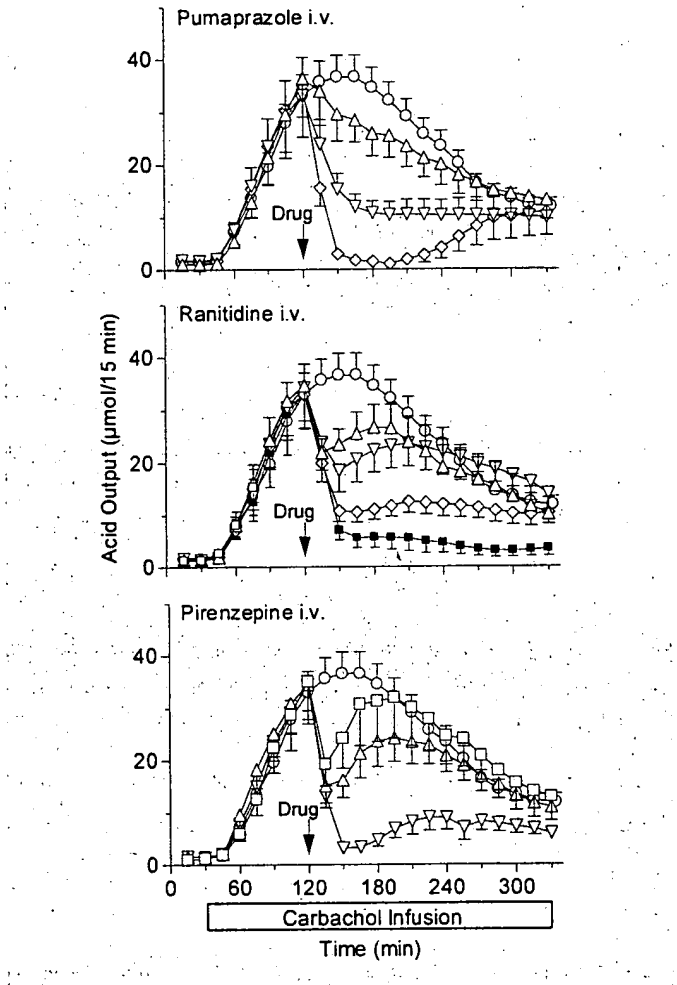


Fig. 5. Acid output (μmol/15 min; mean ± SEM) in the Ghosh-Schild rat stimulated by 0.002 μmol × kg^{-1} × min^{-1} carbachol (i.v. infusion). Drug administration by i.v. bolus. For symbols see legend to figure 3.

only at extremely high doses (fig. 6). The only exception was a comparatively good efficacy and potency of pirenzepine against carbachol as the stimulus (fig. 5, 6). Here, pumaprazole, ranitidine and pirenzepine were about equally effective. Basal acid secretion in the Ghosh-Schild rat was inhibited by pumaprazole with a higher efficacy compared to ranitidine (fig. 7).

Acid Secretion and Gastric Lesions in the Modified Shay Rat

Pumaprazole displayed lower ID_{50} values than ranitidine when tested in the modified Shay rat (fig. 6). This holds true both with respect to inhibition of acid output and inhibition of gastric lesions. Depending on the route of administration, pirenzepine displayed either slightly lower or slightly higher ID_{50} values than ranitidine, but was in any case less potent than pumaprazole (fig. 6). Pumaprazole displayed identical ID_{50} values on day 1 (11 μmol/kg, 95% confidence limits of 5 and 23) and on day 7 (10 μmol/kg, 95% confidence limits of 4 and 23) of a repeated dose study in this model (treatment periods of either 1 or 7 days were studied in separate groups of animals; data not shown).

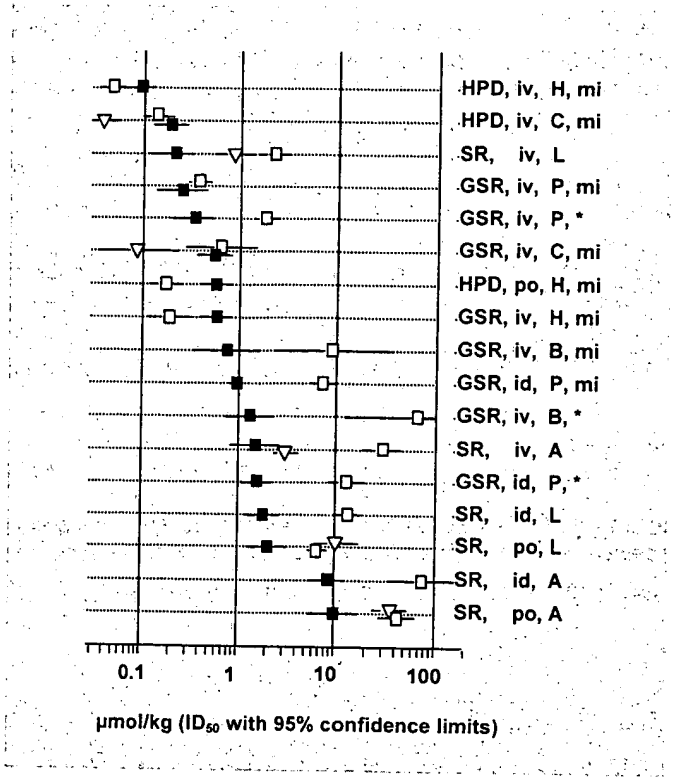


Fig. 6. Inhibition of acid output by pumaprazole (■), ranitidine (□) and pirenzepine (▽) in the Heidenhain pouch dog (HPD), the Ghosh-Schild rat (GSR) and the modified Shay rat (SR). In the modified Shay rat, inhibition of mucosal lesions by the test drugs has been additionally determined. The figure indicates the ID_{50} values (µmol/kg) with 95% confidence limits. The models have been ordered according to increasing ID_{50} values of pumaprazole. The secretagogues used in the animal models are indicated in the figure, except for the Shay rat model (no exogenous secretagogue). Note that the route of administration indicated to the right of the model refers to the test drug, not the secretagogue. * In the Ghosh-Schild rat, the AUCs of the acid-output curves over 3.5 h have been used to calculate mean inhibition of acid output, in addition to maximum inhibition (mi) as indicated in the Method's section. In the Shay rat, inhibition of either acid output (A) or lesion index (L) was measured. All the other animal models only determine acid output, and therefore either the stimulus of acid secretion (H = histamine, C = carbachol, P = pentagastrin) or the basal condition (B; no exogenous secretagogue) is specified. The number of independent experiments was 4–32, depending on dose and model.

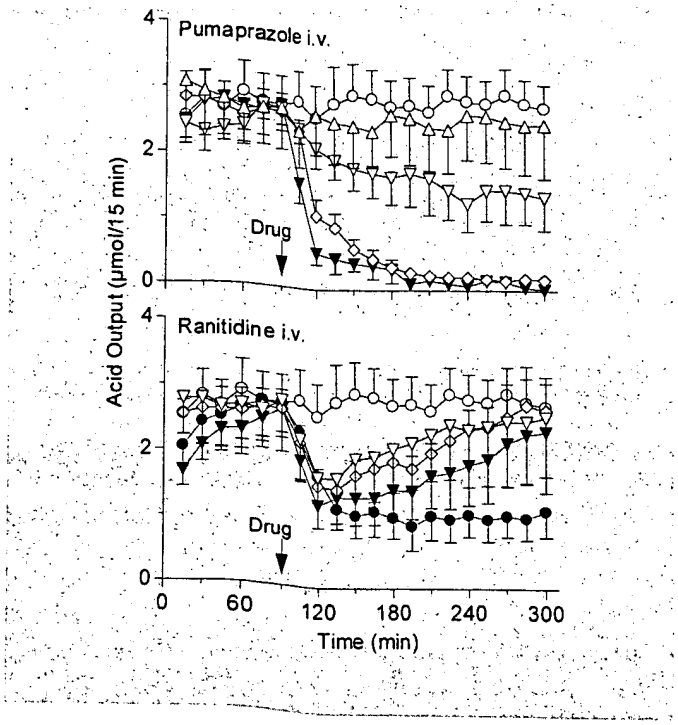


Fig. 7. Acid output (µmol/15 min; mean ± SEM) in the Ghosh-Schild rat, basal secretion (not exogenously stimulated). For symbols see legend to figure 3.

Acid Secretion in the Heidenhain Pouch Dog

In the Heidenhain pouch dog stimulated for acid secretion by histamine, the H_2-receptor antagonist ranitidine displayed slightly lower ID_{50} values than pumaprazole, although the two drugs showed the same efficacy (maximum achievable inhibition of acid output). No difference between ID_{50} values of pumaprazole and ranitidine was found upon carbachol stimulation, under this condition pirenzepine displayed a lower ID_{50} but the same maximum inhibition (efficacy) as pumaprazole. The ID_{50} values are shown in figure 6.

pH Metry in the Fasted, Gastric Fistula Dog

A comparison between oral administrations of the APA pumaprazole, the H_2-receptor antagonist ranitidine, and the PPI omeprazole proved pumaprazole to be the most efficacious one of the three drugs tested (fig. 8). The lag phases between oral administration of the test drugs and the onset of the pH-elevating effects is by the most part due to a delayed gastric emptying of residual acid. Therefore, drug effects have to be compared on the basis of the steepness and the degree of the pH elevation. The lower dose of pumaprazole (27 µmol/kg) rapidly elevated luminal pH up to almost neutrality, the higher dose (54 µmol/kg) further prolonging this pH-elevating effect. By contrast, comparable doses of ranitidine resulted in an

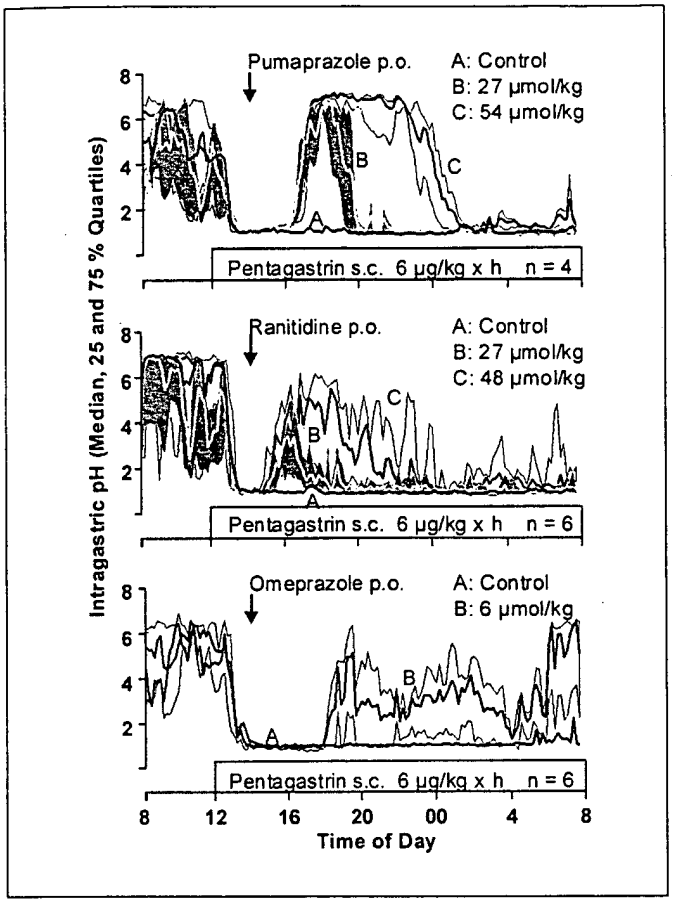


Fig. 8. pH metry in the fasted, gastric fistula dog. Acid secretion was stimulated by a continuous, subcutaneous pentagastrin infusion, 6 µg × kg^{-1} × h^{-1}; n = 4–6. Test drugs have been administered orally at the doses indicated in the figures. All doses are single doses, i.e., first administrations to the animals. Shaded areas and thin lines indicate the 25 and 75% quartiles of the median curves (heavy lines).

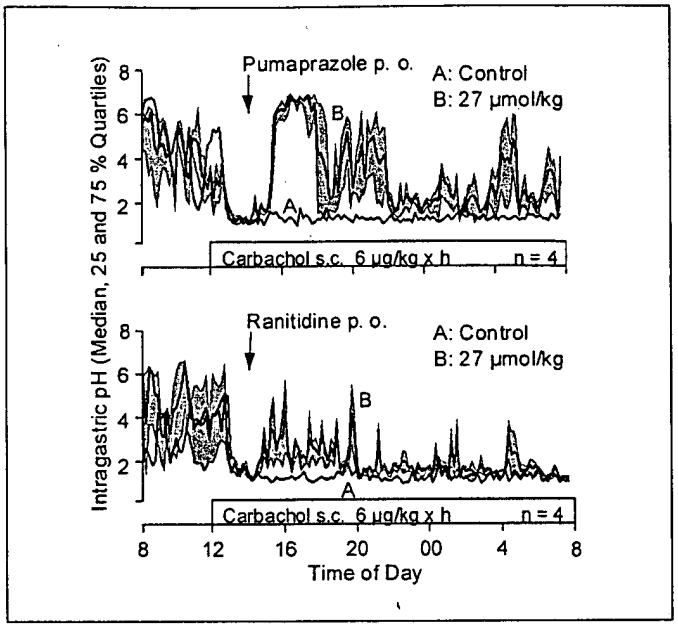


Fig. 9. pH metry in the fasted, gastric fistula dog. Acid secretion was stimulated by a continuous, subcutaneous carbachol infusion, 6 µg × kg^{-1} × h^{-1}; n = 4. Test drugs have been administered orally at 27 µmol/kg. Single doses, i.e., first administrations to the animals. Shaded areas and thin lines indicate the 25 and 75% quartiles of the median curves (heavy lines).

only moderate pH elevation, and the variation in this effect was extremely high compared to the low variation in the effect of pumaprazole. It should be noted that the dose of 27 µmol/kg of ranitidine corresponds to about 4 single clinical doses, and the dose of 48 µmol/kg to about 8 single doses in the patient, when calculated on the basis of mg/70 kg. As to omeprazole, its pH-elevating effect will of course increase during repeated administration due to its covalent mode of interaction with the proton pump. When acid secretion was stimulated by carbachol and intragastric pH was continuously recorded (fig. 9), pumaprazole was again clearly superior to ranitidine in elevating intragastric pH.

Discussion

The present animal data show that a potassium-competitive APA results in an immediate and strong inhibition of acid output and a pronounced elevation of intragastric pH up to almost neutrality even upon a single administration to fasted, pentagastrin-stimulated dogs. APAs block even resting proton pumps [21], and the duration of their antisecretory effect directly depends on the drug's serum concentration because of their reversible mode of action [18]. Therefore, APAs allow optimal control of pH elevation with respect to both its duration and degree.

By contrast, PPIs are pro-drugs that require acid-dependent activation. Because a varying percentage of parietal cells is in the resting state at any time of drug administration, PPIs require 2–3 days of pharmacodynamic accumulation until steady state. It should be noted in this context that pH elevations between 5 and 6 found upon a single oral administration of the PPI lansoprazole to man [34] were predominantly due to the buffering effect of food. As opposed to the weak pH elevation by a single dose of a PPI, its antisecretory effect is nevertheless

strong, and its duration by far outlasts the kinetics of serum concentrations due to the covalent mode of action.

In a comparison between different classes of antisecretory drugs, the efficacy (maximum inhibition) is more important than the potency (ID_{50} value) because there is no rationale for any comparison on a milligram basis between drugs with different mechanisms of action. Our data demonstrate that ranitidine [35], as an example of H_2-receptor antagonists, caused an only moderate and highly variable pH elevation in the gastric lumen of the fasted gastric fistula dog, as opposed to the APA pumaprazole. We recently found that ranitidine was even less effective when its pH-elevating effect within the mucous layer of the fistula dog was compared to the strong effect of an APA [36].

Pumaprazole displayed a similar efficacy as ranitidine against histamine in the Ghosh-Schild rat, and the two antisecretory drugs showed comparable potencies against carbachol stimulation (fig. 6). Actually, in the mouse isolated stomach, cholinergic stimulation of acid secretion was found to be mediated by histamine released from paracrine cells [37, 38]. Histamine released from enterochromaffin-like cells may play a central role for gastric acid secretion also in man [39, 40].

In conclusion, the new therapeutic principle of reversible antagonism of the gastric acid pump by APAs allows an immediate and strong inhibition of acid secretion. By adjusting the dose, this more or less neutralizing effect can either be limited to an appropriate portion of the 24-hour period, or can on demand be extented to the whole 24-hour period. In contrast to PPIs, the maximum effect of a given dose of an APA will already be achieved upon a first administration.

Acknowledgement

We thank Walter Prinz, Uwe Bräuer and Anke Donat for their excellent and expert technical assistance.

References

1 Burget DW, Chiverton SG, Hunt RH: Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. Gastroenterology 1990;99:345–351.

2 Hunt RH: Hp and pH: Implications for the eradication of *Helicobacter pylori*. Scand J Gastroenterol 1993;28(suppl 196):12–16.

3 Axon ATR: The role of acid inhibition in the treatment of *Helicobacter pylori* infection. Scand J Gastroenterol 1994;29(suppl 201):16–23.

4 Klinkenberg-Knol EC, Festen HPM, Meuwissen SGM: Pharmacological management of gastro-oesophageal reflux disease. Drugs 1995; 49:695–710.

5 Berstad K, Berstad A: Review. *Helicobacter pylori* infection in peptic ulcer disease. Scand J Gastroenterol 1993;28:561–567.

6 Marshall BJ: Managing acid peptic disease in the *Helicobacter pylori* era. J Clin Gastroenterol 1995;21(suppl 1):155–159.

7 Black JW, Duncan WAM, Durant CJ, Ganellin CR, Parsons EM: Definition and antagonism of histamine H_2-receptors. Nature 1972;236: 385–390.

8 Hersey SJ, Sachs G: Gastric acid secretion. Physiol Rev 1995;75:155–189.

9 Smith JTL, Gavey C, Nwokolo CU, Pounder RE: Tolerance during 8 days of high-dose H_2-blockade: Placebo-controlled studies of 24-hour acidity and gastrin. Aliment Pharmacol Ther 1990;4(suppl 1):47–63.

10 Nwokolo CU, Prewett EJ, Sawyerr AM, Hudson M, Lim S, Pounder RE: Tolerance during 5 months of dosing with ranitidine, 150 mg nightly: A placebo-controlled, double-blind study. Gastroenterology 1991;101:948–953.

11 Wilder-Smith CH, Merki HS: Tolerance during dosing with H_2-receptor antagonists. An overview. Scand J Gastroenterol 1992;27 (suppl 193):14–19.

12 Bianchi Porro G, Parente F: Duodenal ulcers resistant to H_2 blockers: An emerging therapeutic problem. Scand J Gastroenterol 1988;23 (suppl. 153):81–88.

13 McTavish D, Buckley MM-T, Heel RC: Omeprazole. An updated review of its pharmacology and therapeutic use in acid-related disorders. Drugs 1991;42:138–170.

14 Huber R, Kohl B, Sachs G, Senn-Bilfinger J, Simon WA, Sturm E: Review article: The continuing development of proton pump inhibitors with particular reference to pantoprazole. Aliment Pharmacol Ther 1995;9:363–378.

15 Kromer W: Similarities and differences in the properties of substituted benzimidazoles: A comparison between pantoprazole and related compounds. Digestion 1995;56:443–454.

16 Fitton A, Wiseman L: Pantoprazole. A review of its pharmacological properties and therapeutic use in acid-related disorders. Drugs 1996;51:460–482.

17 Glise H, Martinson J, Solhaug JH, Carling L, Unge P, Engström G, Hallerbäck B: Two and four weeks' treatment for duodenal ulcer. Symptom relief and clinical remission comparing omeprazole and ranitidine. Scand J Gastroenterol 1991;26:137–145.

18 Pope AJ, Parsons ME: Reversible inhibitors of the gastric H^+/K^+-transporting ATPase: A new class of anti-secretory agent. Trends Pharmacol Sci 1993;14:323–325.

19 Mendlein J, Sachs G: Interaction of a K^+-competitive inhibitor, a substituted imidazo[1,2a]pyridine, with the phospho- and dephosphoenzyme forms of H^+,K^+-ATPase. J Biol Chem 1990;265:5030–5036.

20 Munson KB, Gutierrez C, Balaji VN, Ramnarayan K, Sachs G: Identification of an extracytoplasmic region of H^+,K^+-ATPase labeled by a K^+-competitive photoaffinity inhibitor. J Biol Chem 1991;266:18976–18988.

21 Munson KB, Sachs G: Inactivation of H^+,K^+-ATPase by a K^+-competitive photoaffinity inhibitor. Biochemistry 1988;27:3932–3938.

22 Ghosh MN, Schild HO: Continuous recording of acid gastric secretion in the rat. Br J Pharmacol 1958;13:54–61.

23 Metzler CM, Elfring GL, McEwen AJ: A User's Manual for NONLIN and Associated Programs. Kalamazoo, Upjohn, 1974.

24 Unkelbach HD, Wolf T: Qualitative Dosis-Wirkungsanalysen. Stuttgart, Fischer, 1985, pp 13–14.

25 Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H: A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 1945;5:43–61.
26 Okabe S, Takeuchi K, Nakamura K, Takagi K: Pathogenesis of gastric lesions induced by aspirin in the pylorus-ligated rat. Jpn J Pharmacol 1974;24:363–371.
27 Heidenhain R: Über die Absonderung der Fundusdrüsen des Magens. Arch Ges Physiol 1879; 19:148–167.
28 Gregory RA, Tracy HJ: Secretory responses of denervated gastric pouches. Am J Dig Dis 1960;5:308–323.
29 Postius S, Bräuer U: Loss of circadian rhythm in luminal acidity of canine stomach by implantation of a gastric fistula. Digestion 1995; 56:35–40.
30 Postius S, Bräuer U, Kromer W: The novel proton pump inhibitor pantoprazole elevates intragastric pH for a prolonged period when administered under conditions of stimulated gastric acid secretion in the gastric fistula dog. Life Sci 1991;49:1047–1052.
31 McLauchlan G, Rawlings JM, Lucas ML, McCloy RF, Crean GP, McColl K: Electrodes for 24 h pH monitoring: A compartative study. Gut 1987;28:935–939.
32 Bauerfeind P, Duroux Ph, Bumm R, Emde C, Blum AL: Fehlerquellen und Standardisierung intragastraler pH-Metrie. Z Gastroenterol 1988;26(suppl 1):12–19.
33 Hollander M, Wolfe DA (eds): Nonparametric Statistical Methods. New York, Wiley, 1973, pp 75–82.
34 Bell NJV, Hunt RH: Time to maximum effect of lansoprazole on gastric pH in normal male volunteers. Aliment Pharmacol Ther 1996;10: 897–904.
35 Brogden RN, Carmine AA, Heel RC, Speight TM, Avery GS: Ranitidine: A review of its pharmacology and therapeutic use in peptic ulcer disease and other allied diseases. Drugs 1982;24:267–303.
36 Postius S, Bräuer U, Kromer W: The acid pump antagonist (APA) B9106–086 causes a stronger and more reliable pH-elevation in the gastric mucus-layer than ranitidine. Gastroenterology 1997;112(suppl):A261.
37 Black JW, Shankley NP: Pharmacological analysis of the muscarinic receptors involved when McN-A 343 stimulates acid secretion in the mouse isolated stomach. Br J Pharmacol 1985; 86:609–617.
38 Kromer W, Baron E, Boer R, Eltze M: Telenzipine inhibits electrically-stimulated, acetylcholine plus histamine-mediated acid secretion in the mouse isolated stomach by blockade of M_1 muscarine receptors. Naunyn Schmiedebergs Arch Pharmacol 1991;347:7–13.
39 Hakanson R, Sundler F: Histamine-producing cells in the stomach and their role in the regulation of acid secretion. Scand J Gastroenterol 1991;26(suppl 180):88–94.
40 Waldum HL, Brenna E, Kleveland PM, Sandvik AK, Syversen U: The use of gastric acid-inhibitory drugs: Physiological and pathophysiological considerations. Aliment Pharmacol Ther 1993;7:589–596.

0022-3565/90/2541-0129$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 254, No. 1
Printed in U.S.A.

BY 1023/SK&F 96022 INN pantoprazole, a Novel Gastric Proton Pump Inhibitor, Potently Inhibits Acid Secretion But Lacks Relevant Cytochrome P450 Interactions

W. KROMER, S. POSTIUS, R. RIEDEL, W. A. SIMON, G. HANAUER, U. BRAND, S. GÖNNE and M. E. PARSONS

Byk Gulden Pharmaceuticals (W.K., S.P., R.R., W.A.S., G.H., U.B., S.G.), D-7750 Konstanz, FRG and Smith Kline & French Research Ltd. (M.E.P.), The Frythe, Welwyn, Herts AL6 9AR, UK

Accepted for publication March 28, 1990

ABSTRACT

The novel H^+/K^+-adenosine triphosphatase inhibitor (gastric proton pump inhibitor), BY 1023/SK&F 96022, was found to be more potent than omeprazole in some rat models and slightly less potent in a dog model. Overall, both compounds are of a similar potency and efficacy. BY 1023/SK&F 96022 exhibited a somewhat longer duration of the antisecretory action than omeprazole in the Ghosh-Schild rat. In the modified Shay rat, on the basis of equieffective doses in terms of the initial effect, both compounds had a comparable duration of action. However, the p.o./i.v. dose ratio upon acute administration was larger for omeprazole, possibly reflecting its lower stability in the acidic environment of the secreting stomach, compared to BY 1023/SK&F 96022. As *in vivo*, both compounds were equipotent to inhibit acid production in rabbit isolated fundic glands. However, omeprazole interacted with the 7-ethoxycoumarin dealkylase *in vitro* with high affinity (K_i = 38.5 μmol/l), in contrast to BY 1023/SK&F 96022 (K_i = 135 μmol/l). Compared to omeprazole, BY 1023/SK&F 96022 also showed less interaction with the cytochrome P450 enzyme hydroxylating lonazolac. Moreover, this difference between the two compounds was also found in the rat *in vivo* with respect to their interaction with diazepam. Thus, both compounds displayed a comparable antisecretory potency *in vivo* and *in vitro* but showed a different interference with cytochrome P450 in favor of less interaction by BY 1023/SK&F 96022.

Gastric acid secretion by parietal cells is achieved by an enzyme, H^+/K^+-ATPase, which pumps protons in exchange for potassium ions across the apical membrane (Wallmark *et al.*, 1985). A new class of antiulcer drugs, *i.e.*, substituted benzimidazoles like omeprazole (fig. 1b), bind covalently to this enzyme (Fryklund *et al.*, 1988) and inhibit it (Im *et al.*, 1985). In principle, all of the substituted benzimidazoles act by the same mechanism. The parent compound, *i.e.* the benzimidazole sulphoxide, is transformed chemically (activated) in an acidic environment into a cyclic sulfenamide which then reacts covalently with a cysteine thiol group of the H^+/K^+-ATPase (Figala *et al.*, 1986; Lorentzon *et al.*, 1985; Rackur *et al.*, 1985; Senn-Bilfinger *et al.*, 1987; Sturm *et al.*, 1987).

In contrast to receptor antagonists, proton pump inhibitors block the final step in acid production. Because of the resulting strong and long lasting inhibition of gastric acid secretion, these H^+/K^+-ATPase inhibitors offer significant advantages in ulcer therapy over conventional drugs, especially in the most troublesome conditions like Zollinger-Ellison-Syndrome (McArthur *et al.*, 1985). A novel proton pump inhibitor, BY 1023/SK&F 96022 (fig. 1a), proved to be similarly potent to omeprazole in inhibiting gastric acid secretion in healthy male volunteers (Simon *et al.*, 1989). Because, however, therapeutic doses of omeprazole affect oxidative drug metabolism in humans *in vivo* (Clissold and Campoli-Richards, 1986; Diaz *et al.*, 1989; Gugler and Jensen, 1985) and in human hepatocytes *in vitro* (Diaz *et al.*, 1989), we directly compared the two drugs with respect to both their antisecretory/antiulcer effect and inhibitory effect on cytochrome P450, in both respects *in vitro* as well as *in vivo*.

Received for publication November 21, 1989.

Methods

Rat studies: gastric lesions and acid secretion in the modified Shay rat. The experimental procedure of Shay *et al.* (1945), modified by Okabe *et al.* (1974), was used. The pylorus of female Sprague-Dawley rats (150–220 g b.wt., fasted for 24 hr) was ligated under ether anesthesia, the abdomen was closed and 100 mg/kg of ASA in 10 ml/kg were given p.o. Subsequently, the test substance or vehicle was given i.v. or i.d., in 1 ml/kg in either case. Oral drug administration (in 10

ABBREVIATIONS: ATPase, adenosine triphosphatase; ASA, acetylsalicylic acid; i.d., intraduodenally; GSR, Ghosh-Schild rat; AUC, area under the time-response curve; FR, fistula rat; 2-DG, 2-deoxy-D-glucose; rt, running time; db, dibutyryl; cAMP, cyclic AMP; AP, aminopyrine; HPLC, high-performance liquid chromatography; HPD, Heidenbain-pouch dog.

ml/kg) was 1 hr before pylorus ligation. Four hours after pylorus ligation, the stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed, centrifuged, the volume measured and the acidity determined by titration with 0.01 N NaOH to pH 7. The mucosa was flushed with saline and the stomach pinned on a cork plate. The number and size of mucosal lesions were evaluated blind as to drug treatment using a stereomicroscope with 10-fold magnification. A 6-point score was used to assess the diameter of the lesions: 0 = no ulceration; 1 = 0.1 to 1.4 mm; 2 = 1.5 to 2.4 mm; 3 = 2.5 to 3.4 mm; 4 = 3.5 to 4.4 mm; 5 = 4.5 to 5.4 mm; and 6 > 5.4 mm. For each lesion score observed in a particular animal, the number of lesions was counted and this number multiplied by the corresponding score.

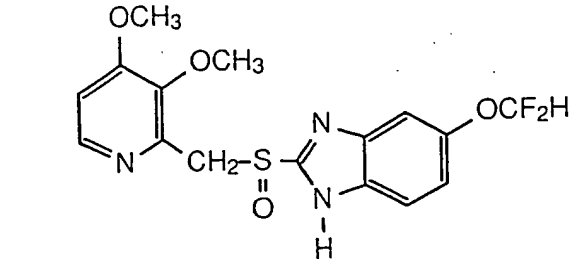
For assessment of duration of antiulcer and antisecretory action in the modified Shay rat, test compounds were administered p.o. 1, 6 or 24 hr before pylorus ligature and the experiments performed as described above.

Acid secretion in the lumen-perfused rat stomach *in situ* (GSR). These experiments were done according to the procedure described by Ghosh and Schild (1958). Female Sprague-Dawley rats, 180 to 260 g b.wt., deprived of food for 24 hr before the experiment with free access to water, were anesthetized with urethane (1.5 g/kg i.m.) and tracheotomized. After a midline abdominal incision, a PVC tube was inserted into the stomach *via* the esophagus and the stomach was perfused with saline (37°C) at a rate of 0.5 ml/min. A second tube draining the pylorus was inserted through the abdominal wall for collection of gastric secretion. Acid secretion was determined at 15-min intervals by titration of the perfusate with 0.01 N NaOH to pH 7. Gastric secretion was stimulated during a 4.5-hr period by an i.v. infusion of 1 $\mu g \times kg^{-1} \times min^{-1}$ pentagastrin starting 30 min after determination of two basal values of acid secretion. This dose of pentagastrin results in 80% of the maximum acid secretion with the advantage of providing a fairly stable plateau over the whole experiment. The test substance was administered i.v. in a volume of 1 ml/kg 60 min after commencement of the pentagastrin stimulation. The dose-response relationship was established using the AUC from 60 to 300 min after drug administration.

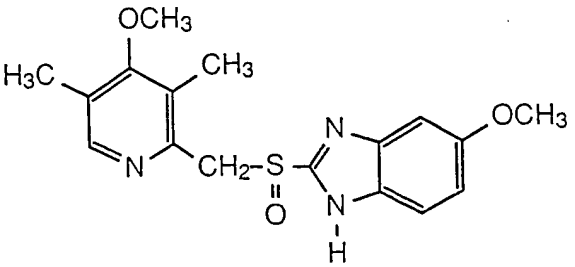
Acid secretion in the acute gastric FR. Female Sprague-Dawley rats, 190 to 240 g b.wt., were deprived of food 16 hr before the experiment with free access to water. Oral administration of the test compound was done by gavage 15 min before anesthesia (urethane, 1.25 $g \times kg^{-1}$ i.m.). After a midline abdominal incision, a PVC catheter was inserted from the duodenum into the stomach, the stomach flushed with saline and the gastric juice drained in 30-min samples. In case of 2-DG stimulation of acid secretion, the rats were adrenalectomized in order to prevent counterregulation of central vagal stimulation by 2-DG (Colin-Jones and Himsworth, 1969). This allows lower 2-DG-doses than otherwise required. The stimulus, *i.e.*, either s.c. bethanechol (0.75 mg/kg) or i.v. 2-DG (50 mg/kg), was administered 45 min after onset of anesthesia. Intravenous administration of test compounds was 30 min after stimulus (in contrast to p.o. administration, see above). Thirty-minute samples of gastric juice were titrated for acid output with 0.1 N NaOH to pH 7. ID_{50} values of test compounds were calculated on the basis of AUC values of the time-response curves for each inhibitor dose, covering a 4-hr period after stimulation in case of p.o. administration of test drug, and a 3-hr period after administration of test drug in case of its i.v. administration.

Dog studies. Male Beagle dogs, aged 2 to 8 years, with Heidenhain pouch (modified after Gregory and Tracy, 1960) were used. Their body weight was between 15 to 19 kg. The animals were kept at a 12-hr light-dark rhythm, housed singly. They received standard tinned food once daily at 10:00 A.M. and tap water *ad libitum*. Measurement of acid output was performed during a 4-hr test period in 15-min fractions. After a basal period of 30 min, gastric secretion was stimulated by continous i.v. infusion of impromidine ($2 \mu g \times kg^{-1} \times hr^{-1}$). This results in a 50% stimulation of maximum acid secretion. Higher doses caused cardiac side effects in a dose-dependent manner. Upon achievement of a stable plateau of acid secretion (90 min), the test substance was administered i.v. (0.1 ml/kg). Mean gastric acid output was determined for a further 2-hr test period. The dose-response relationship was established using the AUC values obtained for each individual dose 60 to 120 min after drug administration.

Interaction with diazepam in the rat *in vivo*. Female Sprague-Dawley rats (170–200 g b.wt.) were put on the upper end of a rubberized bar (diameter 28 mm), hanging at an angle of 45°. The rats had to run a distance of 1 m from the top of the bar to their home cage (bottom). After a training period of 2 hr, all of the rats had a running time of less than 10 sec. Diazepam (4 mg/kg) was administered s.c. 60 min after p.o. administration of the test substance (for doses, see fig. 8), and the running time measured every 30 min for 3 hr, starting 30 min after diazepam administration. Further measurements were performed at hourly intervals until the end of the diazepam effect. A score system was used for evaluation: 0 = rat falls from the bar; 1 = rt > 15 sec (cut off time = 30 sec); 2 = rt 11 to 15 sec; 3 = rt 6 to 10 sec; 4 = rt 0 to 5 sec. For each time point, the score sum in the diazepam-treated group was plotted against time. The intersection of this curve with the minimum individual score in drug-free controls was taken as the duration of action of diazepam. The duration of action of diazepam under the influence of the H^+/K^+-ATPase inhibitor was assessed



a) BY 1023 / SK&F 96022



b) omeprazole

Fig. 1. Chemical structure of BY 1023/SK&F 96022 INN pantoprazole (a) and omeprazole (b). BY 1023/SK&F 96022 is 5-(difluoromethoxy)-2-[[3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole.

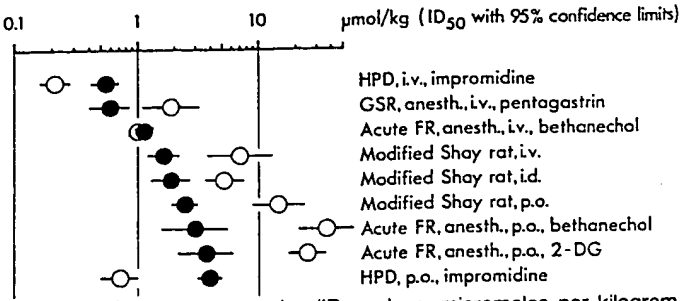


Fig. 2. Antisecretory potencies (ID_{50} values, micromoles per kilogram, with 95% CL) of BY 1023/SK&F 96022 (●) and omeprazole (○) in a variety of rat and dog *in vivo* models. anesth., anesthetized; n = 4–32, depending on the model. The route of administration indicated in the figure refers to the H^+/K^+-ATPase inhibitor, not the stimulus of acid secretion.

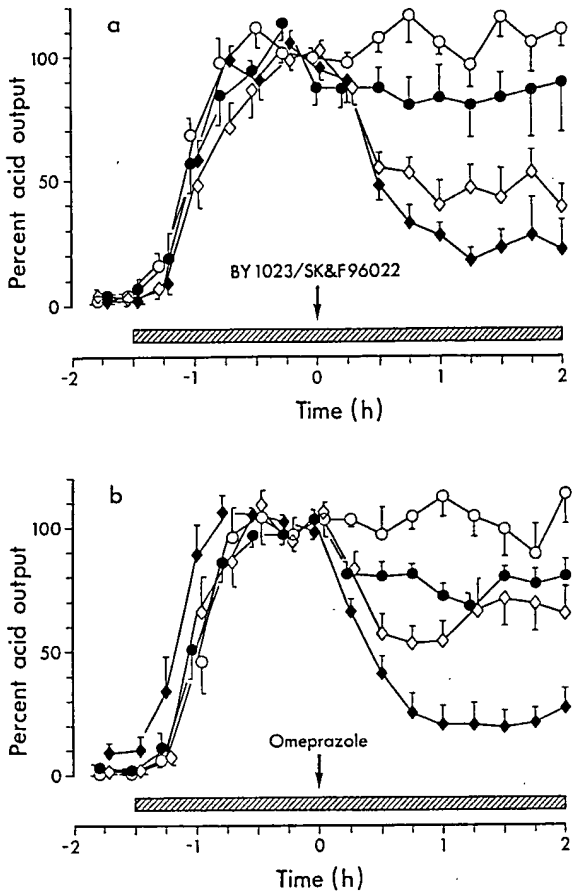


Fig. 3. Percentage of acid output (mean ± S.E.M.) in the conscious HPD under the influence of BY 1023/SK&F 96022 (a) and omeprazole (b), compared to predrug values set at 100%. The horizontal bar indicates continuous stimulation of acid secretion by i.v. impromidine infusion, 2 $\mu g \times kg^{-1} \times h^{-1}$, the vertical arrow indicates i.v. administration of the test compound. Note the dose dependent and long lasting inhibition of acid secretion. Doses (micromoles per kilogram i.v.): 0.33 (●); 0.65 (◇) and 1.3 (◆) for BY 1023/SK&F 96022 (a; $n = 6$) and 0.09 (●); 0.18 (◇) and 0.35 (◆) for omeprazole (b; $n = 5$). Drug-free controls, saline (○), $n = 5$–6. 100% acid output in the different dose groups was, on average, 0.88 ± 0.08 (mmol of H^+/15 min; mean ± S.E.M.). h, hour.

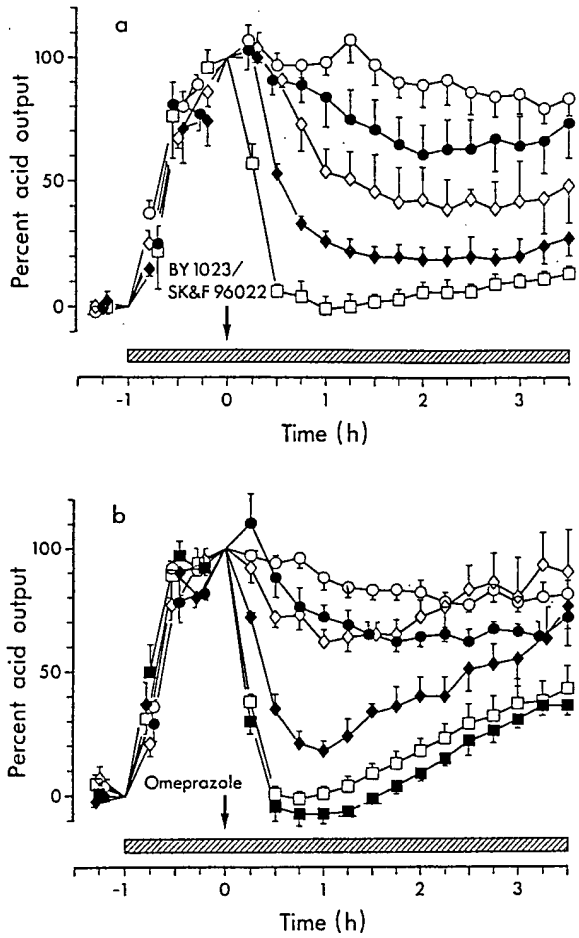


Fig. 4. Percentage of acid secretion (mean ± S.E.M.) in the anesthetized, lumen-perfused rat (GSR) by BY 1023/SK&F 96022 (a) and omeprazole (b), compared to control values set at 100%. The horizontal bar indicates continuous stimulation of acid secretion by i.v. pentagastrin infusion, 1 $\mu g \times kg^{-1} \times min^{-1}$; the vertical arrow indicates i.v. administration of the test compound. Doses (micromoles per kilograms i.v.): 0.3 (●); 0.6 (◇); 1 (◆); 3 (□); 10 (■; only for omeprazole); $n = 4$–6 (test drugs) and 10–11 (○; saline). Note the dose-dependent inhibition of acid secretion, which lasted longer after BY 1023/SK&F 96022 than after omeprazole; 100% acid output in the different dose groups was, on average, 25 ± 1 (μmol of H^+/15 min; mean ± S.E.M.). h, hour.

similarly for each individual dose, and the prolongation of the diazepam action under the influence of omeprazole or BY1023/SK&F 96022 expressed as a percentage of the duration of action of diazepam alone. It should be noted that there was no statistically significant difference between the score sums in totally drug-free *vs.* test drug-treated but diazepam-free controls, *i.e.*, the proton pump inhibitors had no effect of their own (fig. 9). Score sums in these control groups were between 34 and 40.

***In vitro* studies: acid secretion in rabbit isolated gastric glands.** Rabbit fundic glands (White New Zealanders, 2–3 kg b.wt.) were obtained by high-pressure perfusion of the circulation of the stomach and subsequent collagenase treatment of pieces of fundic mucosa. After the glands had been washed several times, they were placed in 20-ml vials with db cAMP (1 mmol/l) and the test compound (3×10^{-8} to 10^{-4} mol/l) in the presence of 0.125 μmol/l [^{14}C]AP and were incubated at 37°C. The incubate was agitated (150 oscillations/min) for 30 min and the reaction stopped by centrifugation (10 sec at 20,000 × *g*). The concentration of radioactivity in supernatant and sediment, normalized to the amount of protein, was used to determine the intracellular accumulation of [^{14}C]AP. This served as an indirect measure of acid secretion, as [^{14}C]AP is a weak base which is trapped in an acidic compartment. For further details, see Kromer *et al.* (1989).

7-Ethoxycoumarin dealkylase activity. In order to induce the

TABLE 1

Extent and duration of the antisecretory action of BY 1023/SK&F 96022 and omeprazole after p.o. administration in the modified Shay rat

Test Compound	p.o. Dose		% Inhibition of Acid Output after a Time Interval between Administration of Test Substance and Pylorus Ligation of:		
			1 hr	6 hr	24 hr
	mg/kg	μmol/kg			
BY 1023/SKF 96022	1.0	2.61	65*	27*	−12 N.S.
	1.5	3.91	64*	42*	19 N.S.
	2.0	5.22	82*	62*	2 N.S.
	3.0	7.82	74*	60*	3 N.S.
	6.0	15.65	84*	71*	12 N.S.
Omeprazole	6.0	20.73	57*	28*	15 N.S.
	8.0	27.63	71*	50*	4 N.S.
	10.0	34.54	79*	57*	12 N.S.
	15.0	51.81	82*	69*	17 N.S
	20.0	69.08	81*	80*	10 N.S.

* $P < .05$, compared to controls; $n = 8$–24.

TABLE 2

Inhibition of lesion index and acid output by BY 1023/SK&F 96022 and omeprazole in the modified Shay rat

Test Compound	ID_{50} with 95% CL					
	Inhibition of lesion index			Inhibition of acid output		
	p.o.	i.d.	i.v.	p.o.	i.d.	i.v.
	μmol/kg				μmol/kg	
BY 1023/SK&F 96022	0.57 (0.39/0.89)	0.79 (0.57/1.10)	0.46 (0.32/0.67)	2.48 (1.90/3.18)	1.89 (1.31/2.73)	1.64 (1.21/2.22)
Omeprazole	7.56 (4.81/11.90)	1.31 (0.84/2.06)	0.90 (0.64/1.27)	14.65 (9.00/23.89)	5.27 (3.63/7.65)	7.01 (3.76/13.03)

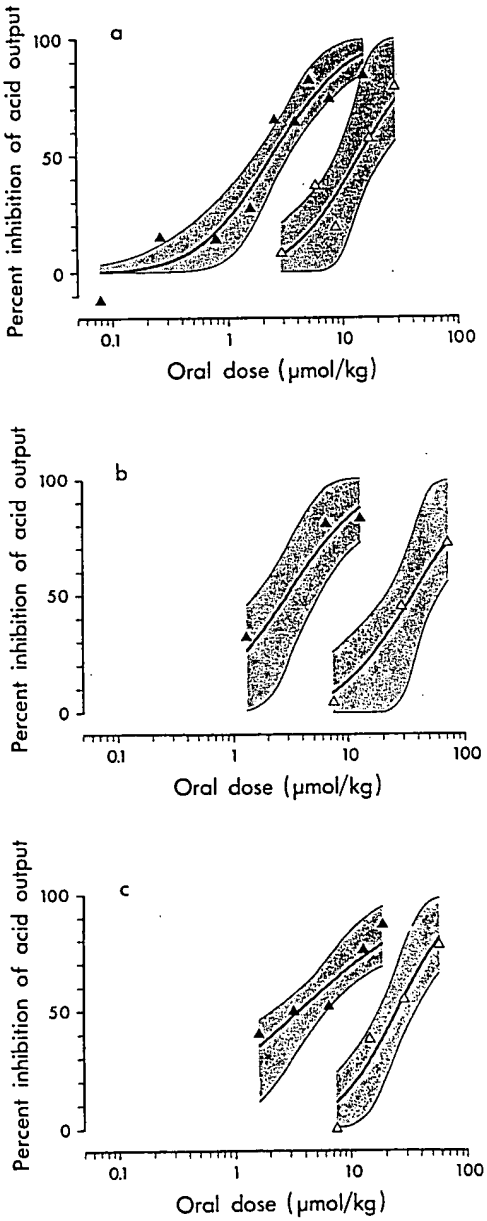


Fig. 5. Dose-response curves of the antisecretory effect (% inhibition) of omeprazole (△) and BY 1023/SK&F 96022 (▲) after p.o. administration to the modified Shay rat (a), and the acute gastric FR upon stimulation of acid secretion by bethanechol (b) or 2-DG (c). Medians and 95% CL; n = 8–24, depending on the model and dose.

TABLE 3

Inhibition of acid output by BY 1023/SK&F 96022 in the anesthetized, acute gastric FR (n = 10)

Test Compound and Route of Administration	Stimulus of Acid Secretion	ID_{50}, μmol/kg (medians; 95% CL)
BY 1023/SK&F 96022		
p.o.	Bethanechol	2.98 (1.57/5.66)
i.v.	Bethanechol	1.14 (0.96/1.35)
p.o.	2-DG	3.65 (2.15/6.20)
Omeprazole		
p.o.	Bethanechol	36.3 (21.4/61.4)
i.v.	Bethanechol	1.03 (0.88/1.19)
p.o.	2-DG	24.9 (17.5/35.5)

TABLE 4

[^{14}C]AP accumulation as an indicator of acid secretion in rabbit isolated fundic glands

Inhibition by BY 1023/SK&F 96022 and omeprazole.

Test Compound	IC_{50}			n
	Mean	95% CL		
		Upper	Lower	
		μmol/l	μmol/l	
BY 1023/SK&F 96022	1.0	3.80	0.27	6
Omeprazole	0.7	3.31	0.14	5

cytochrome P450 system of the liver, female rats (180–200 g b.wt., SIV 50 strain) were given 1 g/l of phenobarbital in the drinking water for 7 days. Their livers were homogenized in 0.1 mol/l of phosphate buffer, pH 7.4 (Potter-Elvejhem, 860 rpm, 6 strokes) and subjected to two-step centrifugation (20 min at 10,000 × g, 60 min at 100,000 × g); the 100,000 × g sediment was stored at −80°C. One milligram of protein was incubated in 100 mmol/l of TRIS-HCl (pH 7.4) in the presence of 10, 15, 30 and 100 μmol/l of 7-ethoxycoumarin plus test compound in a concentration range of 0 to 50 (omeprazole) or 0 to 100 μmol/l (BY 1023/SK&F 96022), respectively. The K_m of 7-ethoxycoumarin is 33 μmol/l. The reaction was started with 0.3 mmol/l of $NADPH_2$. After 15 min of incubation at 37°C, the reaction was terminated by adding in sequence 125 μl of trichloroacetic acid (15% w/v) and 2 ml of chloroform. After vigorous shaking and centrifugation for 5 min, 1 ml of the organic phase was extracted with 2 ml 0.01 N NaOH plus 1 mol/l NaCl, and the concentration of the 7-hydroxycoumarin in the alkaline phase was determined fluorometrically at both 336 (excitation) and 460 nm (emission). Data were evaluated according to Lineweaver and Burk (1934). For further methodological details, see Greenlec and Poland (1978).

Arylhydroxylase activity. Enzyme preparation was as described above. Microsomal protein (0.3 mg/ml) were incubated in 100 mmol/l of TRIS-HCl buffer, pH 7.4, in the presence of lonazolac (25, 50 and 100 μmol/l) and test compound (0–300 μmol/l). The K_m of lonazolac is 25 μmol/l. The reaction was started by addition of $NADPH_2$, final concentration 1 mmol/l. Incubation was at 37°C for 15 min. The reaction was stopped by freezing in liquid nitrogen, samples were then thawed and centrifuged at 20,000 × g to remove protein before analysis

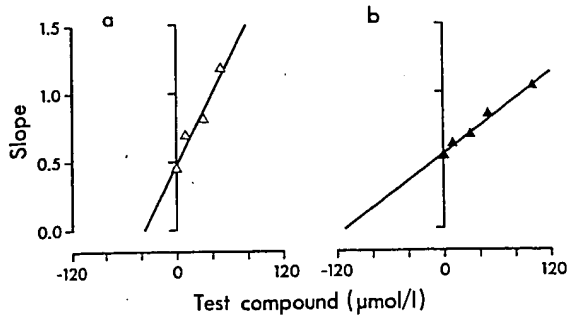


Fig. 6. Examples of the influence of omeprazole (a) and BY 1023/SK&F 96022 (b) on 7-ethoxycoumarin dealkylase from rat liver. Secondary plot according to Lineweaver-Burk: slope *vs.* inhibitor concentration (micromoles per liter). The intersection of the lines with the abscissa provides the numerical value for K_i (mean ± S.E.M.; $n = 4$): 38.5 ± 2.7 (omeprazole) and 135 ± 12.3 μmol/l (BY 1023/SK&F 96022).

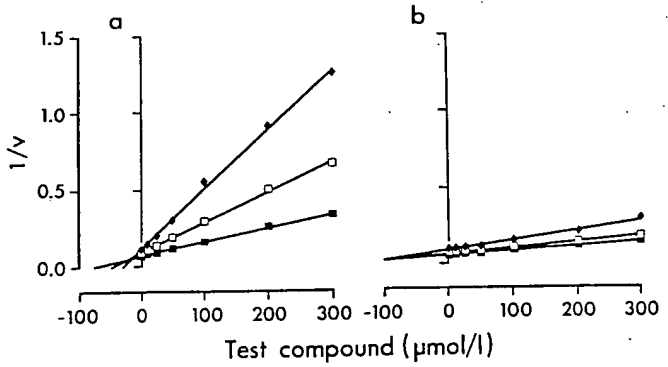


Fig. 7. Examples of the influence of omeprazole (a) and BY 1023/SK&F 96022 (b) on liver arylhydroxylase activity. Dixon Plot of one representative experiment out of four. Ordinate: 1/V; velocity (V) = μmol *p*-hydroxylonazolac × 15 min^{-1} × 0.3 mg of $protein^{-1}$. Abscissa: inhibitor concentration (micromoles per liter). The three lines represent 25 (◆), 50 (□) and 100 (■) μmol/l of substrate (lonazolac). The projection of the intersection of the three lines to the abscissa provides the numerical value (mean ± S.E.M.) of K_i. Omeprazole, 19.5 ± 3.4 μmol/l; BY 1023/SK&F 96022, 113 ± 8 μmol/l. Each K_i value is calculated from the data of four separate experiments, resulting in a total of 12 intersections between two lines each from different substrate concentrations.

on HPLC. HPLC analysis of lonazolac (substrate) and its metabolite, hydroxylonazolac, was performed on a reversed-phase column. Nucleosil C 8.5 μm (column, 125 × 4.6 mm) and LiCHROPREP RP-2 (precolumn, 12 × 4 mm) were used. A gradient of acetonitrile (10–45%) and 10 mmol/l of KH_2PO_4 buffer, pH 7.4, was used as eluent on the analytical column over 15 min. Data were evaluated according to Dixon (1953).

Statistics. In case of the modified Shay rat, the acute FR and the HPD, the average percentage of changes in acid output under drug treatment (median with 95% CL) as compared to separate controls were calculated according to Hodges-Lehmann and Moses (cited in Hollander and Wolfe, 1973). The doses which caused 50% inhibition (ID_{50} values with 95% CL) were interpolated from dose-response curves, and statistical significance was assessed by the above test procedure. In case of the GSR, acid secretion after administration of the test compound (or vehicle) was related to that before administration in the same animal.

Drugs. ASA (Merck, Darmstadt, Germany), dissolved in 1% tylose, pH 3.6; bethanechol hydrochloride (Calaire Chimie S.A., Paris, France); BY 1023/SK&F 96022 (Byk Gulden Pharmaceuticals, Konstanz, Germany), aqueous solution, pH 9–10 (rat studies, all routes of administration, and HPD, i.v. administration), methanolic (0.1%) solution in case of *in vitro* studies; 2-DG (Aldrich Chemical Company Inc., Milwaukee, WI), dissolved in physiological saline; diazepam (Hoffmann-La Roche, Nutley, NJ), Valium-ampoules, further diluted in 20% PEG

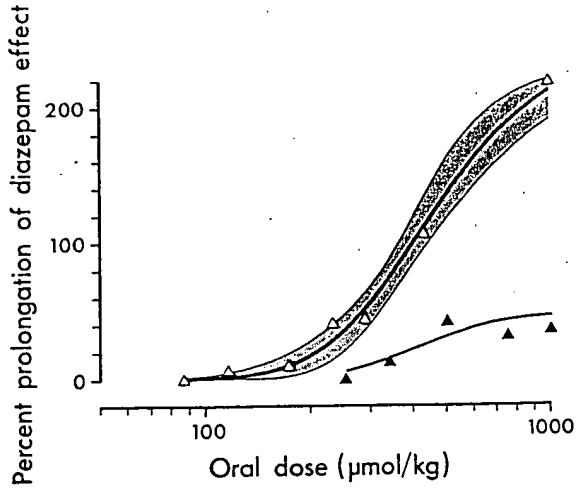


Fig. 8. Influence of omeprazole (Δ) and BY 1023/SK/F 96022 (▲) on motor performance of rats under diazepam treatment, put on a hanging bar. Ordinate: percentage of prolongation of diazepam effect, compared to diazepam-treated controls; percentages were calculated from $n = 10$ independent data. Abscissa: p.o. dose of test drug (micromoles per kilogram). The hatched area indicates the 95% CL. Omeprazole caused a 50% prolongation of the diazepam effect at 272 μmol/kg. In contrast, a shallow dose-response curve resulting in less than 50% prolongation of the diazepam effect at the extremely high dose of, roughly, 1000 μmol/kg, was found for BY 1023/SK&F 96022. Therefore, no CL could be calculated. There is a significantly stronger enhancement by omeprazole, compared to BY 1023/SK&F 96022, of diazepam action.

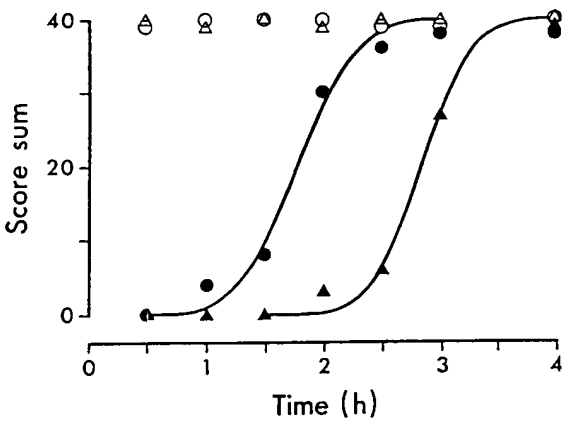


Fig. 9. An example of the prolongation of the diazepam-induced increase in running time of rats on the hanging bar by omprazole, 290 μmol/kg p.o. Ordinate: score sum ($n = 10$); abscissa: time [hr (h)] after s.c. diazepam administration, which was 60 min after p.o. administration of test drug. Symbols: drug-free controls (○); diazepam-treated but omeprazole-free controls (●); omeprazole-treated but diazepam-free controls (Δ); omeprazole plus diazepam-treated rats (▲). Note that omeprazole (and BY 1023/SK&F 96022 alike; not shown), had no effect of its own on rt, compared to drug-free controls. The prolongation of the diazepam effect on rt represents, therefore, an interaction between the two compounds.

400; 7-ethoxycoumarin (Sigma, Chemical Co., St. Louis, MO), aqueous solution with dimethylformamide (<0.1%); impromidine (Smith Kline & French Laboratories, Philadelphia, PA) dissolved in physiological saline; lonazolac (calcium-[3-(*p*-chlorophenyl)-1-phenylpyrazol-4]-acetate; Byk Gulden Pharmaceuticals), methanolic (0.1%) solution; omeprazole (the sample was prepared by our chemistry department), aqueous solution, pH 9–10 (rat and dog studies, all routes of administration), methanolic (0.1%) solution in case of *in vitro* studies; pentagastrin (Merck), aqueous solution, pH 8–8.5; and urethane (Merck). The respective solvents were used for controls.

Results

A direct comparison between the antisecretory potencies (ID_{50} values) of BY 1023/SK&F 96022 and omeprazole in nine rat and dog *in vivo* models is shown in figure 2. The results are more uniform with BY 1023/SK&F 96022 compared to omeprazole. Although both compounds proved equipotent in two rat models (*i.v.* administration in GSR under pentagastrin-stimulation and in acute FR under bethanechol-stimulation), it is nevertheless evident from figure 2 that BY 1023/SK&F 96022 is, overall, more potent in the rat, compared to omeprazole, whereas the reverse is true in the dog. This is independent of the route of administration, *i.e.*, i.v. *vs.* p.o. or i.d. administration. The higher potency of BY 1023/SK&F 96022 in the rat, compared to omeprazole, also does not depend on any interference with anesthesia, inasmuch as the same difference between the test compounds was found in conscious animals, although under different experimental conditions (see "Discussion").

There is no indication of any differences in the duration of action between both drugs in the HPD (fig. 3, a and b). However, BY 1023/SK&F 96022, at an equipotent dose in terms of the immediate effect, clearly acts longer than omeprazole in the GSR (fig. 4). All of the actions of the two drugs are dose-dependent (figs. 3 and 4). In contrast to the GSR (fig. 4), BY 1023/SK&F 96022 and omeprazole show a similar duration of action in the modified Shay rat after p.o. administration (table 1). Here, essentially the same percentage of inhibition of acid output is observed with BY 1023/SK&F 96022 and omeprazole, when equieffective doses chosen on the basis of their effect after administration 1 hr before pylorus ligation, are administered 6 hr before pylorus ligation. In spite of the similarity in duration of action of equieffective p.o. doses, however, the ratio of p.o. over i.v. antisecretory ID_{50} values is considerably higher for omeprazole than for BY 1023/SK&F 96022 (table 2).

A comparison between dose-response curves upon p.o. administration of the two H^+/K^+-ATPase inhibitors in the different rat models is given in figure 5. It indicates once more the significantly higher potency of BY 1023/SK&F 96022, compared to omeprazole. Apart from the overview shown in figure 2, numerical values obtained in the acute FR after both p.o. and i.v. administration are shown in table 3, which again demonstrates a significantly larger p.o./i.v. dose ratio of omeprazole, after bethanechol stimulation, compared to BY 1023/SK&F 96022. This suggests superior bioavailability of the latter drug.

BY 1023/SK&F 96022 and omeprazole inhibited [^{14}C]AP accumulation as stimulated by db-cAMP in rabbit isolated fundic glands with a similar IC_{50} (table 4). Despite the similar antisecretory potency *in vitro*, omeprazole significantly inhibited 7-ethoxycoumarin dealkylase activity *in vitro* with a mean K_i value of 38.5 ± 2.7 (μmol/l; mean ± S.E.M.; $n = 4$; fig. 6a) in comparison to 135 ± 12.3 for BY 1023/SK&F 96022 (fig. 6b). A similar split between the two drugs was found in their interaction with the liver cytochrome P450 enzyme hydroxylating lonazolac (fig. 7). Again, omeprazole showed a significantly more pronounced interaction than BY 1023/SK&F 96022. The K_i values (mean ± S.E.M.; $n = 4$) were 19.5 ± 3.4 and 113 ± 8 μmol/l, respectively.

In the rat *in vivo*, omeprazole and, significantly less so, BY 1023/SK&F 96022 prolonged the influence of diazepam on motor performance (fig. 8), although at p.o. doses well away from the dose range of the antisecretory action (fig. 2). A 50% prolongation of the diazepam effect was observed upon 272 μmol/kg of omeprazole. In contrast, the influence of BY 1023/SK&F 96022 was so weak that, even at the extremely high dose of 1000 μmol/kg, no 50% prolongation was found (fig. 8). Figure 9 gives an example of the time-response curve for 290 μmol/kg of omeprazole in comparison to drug-free controls, diazepam-treated but omeprazole-free controls and omeprazole-treated but diazepam-free controls.

Discussion

The present data demonstrate a similar gastric antisecretory potency of omeprazole and the novel H^+/K^+-ATPase inhibitor BY 1023/SK&F 96022, although the latter compound proved more favorable in terms of undesired cytochrome P450 interaction. It is obvious from figure 2 that both of the proton pump inhibitors potently inhibit gastric acid secretion irrespective of the particular stimulus involved. Thus, acid secretion was blocked under pentagastrin-, bethanechol- and impromidine-stimulation, which covers all of the three excitatory receptors present on parietal cells. Acid secretion in response to 2-DG, which centrally stimulates vagal output to the stomach (Hirschowitz and Sachs, 1965), is also inhibited. Proton pump inhibitors block the final step in acid production and, hence, are the most effective inhibitors of acid secretion known.

In the modified Shay rat (table 2), the ratio of p.o. over i.v. ID_{50} values is considerably higher for omeprazole than for BY 1023/SK&F 96022. In fact, BY 1023/SK&F 96022 (a dialkoxy pyridyl compound) is somewhat more stable than omeprazole (a monoalkoxy pyridyl compound) under moderately acidic conditions (Simon *et al.*, 1988). This may also explain the larger variability between different models, found with omeprazole (fig. 2). Actually, the drugs are degraded to the active and, furthermore, to inactive principle(s) in the acidic compartment of the parietal cell and stomach, respectively, thereby losing efficacy dependent on acid lability of the particular compound. A certain percentage of the parietal cells are in their resting state at the time of drug administration and will, therefore, not be inhibited if the drug is already degraded at the time these parietal cells again start to secrete acid. Therefore, an optimized compound should be stable in a moderately acidic environment in order both, not to get degraded and not to affect cells other than the secreting and, hence, highly acidic parietal cell (selectivity of the drug). Below a pH of about 3, however, the drug should rapidly become activated to block further acid production. BY 1023/SK&F 96022 appears to get close to this optimum (Simon *et al.*, 1988; E. Sturm, in preparation).

The ratio of p.o. over i.v. antisecretory ID_{50} values of 1.5 for BY 1023/SK&F 96022 indicates an excellent bioavailability, whereas a ratio of 2.1 for omeprazole suggests that a somewhat smaller fraction of the p.o. administered dose is eventually pharmacodynamically effective under these experimental conditions. The split between p.o./i.v. ID_{50} ratios of BY 1023/SK&F 96022 and omeprazole is even more pronounced when inhibition of mucosal lesions is considered (table 2). Here, the ratio is 1.2 for BY 1023/SK&F 96022 and 8.4 for omeprazole. Because the difference between the two drugs is much smaller when i.d. and i.v. data are compared, the reason for the relatively poor p.o. potency of omeprazole may relate to the acidic condition in the gastric lumen.

An important issue is the potential interaction of substituted

benzimidazoles with liver cytochrome P450. *In vitro* experments and animal *in vivo* studies now demonstrate a clear cut advantage of BY 1023/SK&F 96022 over omeprazole in this respect. Although both drugs proved equipotent in inhibiting acid secretion in rabbit isolated fundic glands (table 4), omeprazole interacts much more strongly than BY 1023/SK&F 96022 with the 7-ethoxycoumarin dealkylase (fig. 6). The K_i of BY 1023/SK&F 96022 is of the same magnitude as that found by Chenery *et al.* (1988) in a different test system. A similar difference between omeprazole and BY1023/SK&F 96022 was confirmed in another *in vitro* model (arylhydroxylase with lonazolac as the substrate; fig. 7) as well as *in vivo* in rats (fig. 8). The *in vivo* method and its validation will be published in detail elsewhere (G. Hanauer *et al.*, in preparation). Although omeprazole prolongs the diazepam effect only at a dose level in excess of that necessary to achieve inhibition of gastric acid secretion in the rat (see figs. 2 and 8), it nevertheless caused significant inhibition of diazepam elimination when administered in a clinically relevant dose to humans (Diaz *et al.*, 1989; Gugler and Jensen, 1985). Hence, the less pronounced interaction with cytochrome P450 shown by BY 1023/SK&F 96022 may be of clinical advantage even though the dose level in the rat, in terms of absolute values, may not apply to humans. As an alternative explanation, the different dose levels of omeprazole interacting, in the rat and in humans, with cytochrome P450 may correspond to single dosing in our rat experiments as opposed to repeated dosing in the human study of Gugler and Jensen (1985). Whatever the reason may be, the decrease in plasma clearance of diazepam and phenytoin caused by clinically relevant doses of omeprazole in humans (Gugler and Jensen, 1985) indicate that a safety margin as large as possible in this respect is advantageous. It is particularly noteworthy, therefore, that BY 1023/SK&F 96022 was, overall, more potent than omeprazole as an inhibitor of acid secretion in the rat (fig. 2), but proved less potent than omeprazole in prolonging the diazepam effect in the same species (fig. 8).

Acknowledgment

The authors wish to thank Mr. Walter Prinz for producing the illustrations and for expert assistance with statistical evaluation of the data.

References

CHENERY, R. J., AYRTON, A., OLDHAM, H. G., NORMAN, S. J. AND STANDRING, P.: The interaction of omeprazole with rat liver cytochrome P450-mediated monooxygenase reactions *in vitro* and *in vivo*. Biochem. Pharmacol. **37:** 1407–1414, 1988.

CLISSOLD, S. P. AND CAMPOLI-RICHARDS, D. M.: Omeprazole. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in peptic ulcer disease and Zollinger-Ellison Syndrome. Drugs **32:** 15–47, 1986.

COLIN-JONES, D. G. AND HIMSWORTH, R. L.: The secretion of gastric acid in response to a lack of metabolizable glucose. J. Physiol. (Lond.) **202:** 97–109, 1969.

DIAZ, D., DAUJAT, M., FABRE, I., FABRE, P., BORIES, P., MAUREL, P. AND MICHEL, H.: Omeprazole is an inducer of the cytochrome P450 IA subfamily in man: A deleterious action? Gastroenterology **96:** (Suppl.) A593, 1989.

DIXON, M.: The determination of enzyme inhibitor constants. Biochem. J. **55:** 170–171, 1953.

FIGALA, V., KLEMM, K., KOHL, B., KRÜGER, U., RAINER, G., SCHAEFER, H., SENN-BILFINGER, J. AND STURM, E.: Acid activation of (H^+-K^+)-ATPase inhibiting 2-(2-pyridylmethylsulphinyl)benzimidazoles: Isolation and characterization of the thiophilic "active principle" and its reactions. J. Chem. Soc. Chem. Commun. 125–127, 1986.

FRYKLUND, J., GEDDA, K. AND WALLMARK, B.: Specific labelling of gastric H^+,K^+-ATPase by omeprazole. Biochem. Pharmacol. **37:** 2543–2549, 1988.

GHOSH, M. N. AND SCHILD, H. O.: Continuous recording of acid gastric secretion in the rat. Br. J. Pharmacol. **13:** 54–61, 1958.

GREENLEE, W. F. AND POLAND, A.: An improved assay of 7-ethoxycoumarin o-deethylase activity: Induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Pharmacol. Exp. Ther. **205:** 596–605, 1978.

GREGORY, R. A. AND TRACY, H. J.: Secretory responses of denervated gastric pouches. Am. J. Dig. Dis. **5:** 308–323, 1960.

GUGLER, R. AND JENSEN, J. C.: Omeprazole inhibits oxidative drug metabolism. Gastroenterology **89:** 1235–1241, 1985.

HIRSCHOWITZ, B. I. AND SACHS, G.: Vagal gastric secretory stimulation by 2-deoxy-D-glucose. Am. J. Physiol. **209:** 452–460, 1965.

HOLLANDER, M. AND WOLFE, D. A.: Nonparametric Statistical Methods, pp. 75–82, Wiley, New York, 1973.

IM, W. B., BLAKEMAN, D. P. AND DAVIS, J. P.: Irreversible inactivation of rat gastric (H^+-K^+)-ATPase *in vivo* by omeprazole. Biochem. Biophys. Res. Commun. **126:** 78–82, 1985.

KROMER, W., BARON, E., BEINBORN, M., ELTZE, M. AND SIMON, W. A.: Stimulation by McN-A-343 and blockade by telenzepine of acid secretion in the mouse isolated stomach at histamine-liberating cells. Naunyn-Schmiedeberg's Arch. Pharmacol. **340:** 68–75, 1989.

LINEWEAVER, H. AND BURK, D.: The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56:** 658–666, 1934.

LORENTZON, P., EKLUNDH, B., BRÄNDSTRÖM, A. AND WALLMARK, B.: The mechanism for inhibition of gastric (H^++K^+)-ATPase by omeprazole. Biochim. Biophys. Acta. **817:** 25–32, 1985.

MCARTHUR, K. E., COLLEN, M. J., MATON, P. N., CHERNER, J. A., HOWARD, J. M., CIARLEGLIO, C. A., CORNELIUS, M. J., JENSEN, R. T. AND GARDNER, J. D.: Omeprazole: Effective, convenient therapy for Zollinger-Ellison syndrome. Gastroenterology **88:** 939–944, 1985.

OKABE, S., TAKENDI, K., NAKAMURA, K. AND TAKAGI, K.: Pathogenesis of gastric lesions induced by aspirin in the pylorus ligated rat. Jp. J. Pharmacol. **24:** 363–371, 1974.

RACKUR, G., BICKEL, M., FEHLHABER, H. W., HERLING, A., HITZEL, V., LANG, H. J., RÖSNER, M. AND WEYER, R.: 2-((2-pyridylmethyl)sulfinyl)benzimidazoles: Acid sensitive suicide inhibitors of the proton transport system in the parietal cell. Biochem. Biophys. Res. Commun. **128:** 477–484, 1985.

SENN-BILFINGER, J., KRÜGER, U., STURM, E., FIGALA, V., KLEMM, K., KOHL, B., RAINER, G. AND SCHAEFER, H.: (H^+-K^+)-ATPase inhibiting 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles. 2. The reaction cascade induced by treatment with acids. Formation of 5H-pyrido[1′,2′:4,5][1,2,4]thiadiazino[2,3-a]benzimidazol-13-ium salts and their reactions with thiols. J. Org. Chem. **52:** 4582–4592, 1987.

SHAY, H., KOMAROV, S. A., FELS, S. S., MERANZE, D., GRUENSTEIN, M. AND SIPLET, H.: A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology **5:** 43–61, 1945.

SIMON, B., MÜLLER, P., BLIESATH, H., LÜHMANN, R., HARTMANN, M. AND WURST, W.: First administration to man of the dimethoxypyridyl substituted benzimidazole BY 1023/SK&F 96022, a novel inhibitor of the gastric H^+,K^+-ATPase. Gastroenterology **96:** (Suppl) A473, 1989.

SIMON, W. A., STURM, E. AND KOHL, B.: Inhibition of (H^+,K^+)ATPase by 2-((2-pyridyl-methyl)-sulphinyl)-benzimidazoles: Improved pH selectivity of dialkoxypyridyl-derivatives. Gastroenterol. Inter. **1:** Suppl. 1, 1157, 1988.

STURM, E., KRÜGER, U., SENN-BILFINGER, J., FIGALA, V., KLEMM, K., KOHL, B., RAINER, G. AND SCHAEFER, H.: (H^+-K^+)-ATPase inhibiting 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles. 1. Their reaction with thiols under acidic conditions. Disulfide containing 2-pyridiniobenzimidazolides as mimics for the inhibited enzyme. J. Org. Chem. **52:** 4573–4581, 1987.

WALLMARK, B., LARSSON, H. AND HUMBLE, L.: The relationship between gastric acid secretion and gastric H^+,K^+-ATPase activity. J. Biol. Chem. **260:** 13681–13684, 1985.

Send reprint requests to: Professor Dr. med. W. Kromer, Byk Gulden Pharmaceuticals, Byk-Gulden-Straße 2, D-7750 Konsanz, W. Germany

Brit. J. Pharmacol. (1958), 13, 54.

CONTINUOUS RECORDING OF ACID GASTRIC SECRETION IN THE RAT

BY

M. N. GHOSH* AND H. O. SCHILD

From the Department of Pharmacology, University College, London

(RECEIVED SEPTEMBER 13, 1957)

A method is described for the continuous recording of acid gastric secretion in the rat. The stomach of the rat anaesthetized with urethane is perfused with a dilute sodium hydroxide solution by way of the oesophagus and the *p*H of the fluid emerging from a cannula in the pylorus is registered graphically. In passing through the stomach, the perfusate collects sufficient buffer to act as an approximately linear buffer system over the relevant range, so that the change in *p*H becomes a measure of acid secretion. The preparation is suitable for the bioassay of secretory stimulants. Ten or more drug doses can be administered in succession in one preparation, so that each animal serves as a self-contained assay unit. Intravenous doses of histamine, methacholine, carbachol and acetylcholine produce a graded and reversible stimulation of acid secretion. The secretory effect of histamine is markedly and specifically potentiated by antihistaminases.

The effect of drugs on gastric secretion in the rat has usually been tested in the pylorus-ligated preparation (Roe and Dyer, 1939; Komarov, Shay, Rayport, and Fels, 1944; Shay, Komarov, Fels, Meranze, Gruenstein and Siplet, 1945). In this method only one dose of drug is administered to each animal so that large numbers of rats are required for a quantitative assay. In the present paper a continuous recording method is described which is sufficiently sensitive to allow several doses to be administered in succession to the same preparation so that each animal serves as a self-contained assay unit or sub-unit.

Methods for the continuous recording of the *p*H of the gastric contents have previously been described (Flexner and Kniazuk, 1940; Rovelstad, Owen, and Magath, 1952), but they provided no information on the amount of acid secreted. In order to obtain this information it is necessary to use a perfusate which changes its *p*H when acid is secreted. In the present work we used a solution of dilute sodium hydroxide, the *p*H of which changes, when perfused through the stomach of the rat, by up to three units, according to the amount of acid secreted.

As single intravenous injections of drugs produce a measurable secretion of acid in this preparation, it can be used for the bioassay of secretory stimulants. The stimulant effects of histamine and choline esters, and the potentiating effects of antihistaminases, will be described.

A brief description of the continuous recording method has already been published (Ghosh and Schild, 1955).

METHODS

Two uniform breeds of rats were used, male and female albino rats and hooded rats. Their weights ranged from 150 to 400 g., with an average of 190 g. The animals were not starved, and were anaesthetized by a single intramuscular injection of urethane. The dose of urethane in terms of body weight required for different rats varied slightly; the sensitivity to urethane appeared to depend on seasonal influences. We generally used doses of 0.5 to 0.7 ml./100 g. of 25% solution of urethane; when the first dose failed to anaesthetize in 30 to 40 min., an additional intramuscular dose was given. These doses caused no interference with respiration. After the administration of the anaesthetic, the body temperature fell. In most experiments it was artificially stabilized at 30° by means of a rectal contact thermometer which controlled the heating of the operating table through an electronic relay. The operating table was tilted and was heated from below by a 25 watt electric lamp, and from above by two 40 watt strip lights (tube lamps) fitted with reflectors, one on each side of the table. When the body temperature was checked by a thermometer introduced into the vagina it varied by less than ±0.25°.

Operative Technique.—The trachea was exposed and cannulated. A polythene tube of 11 cm. length and 2 mm. external diameter was passed into the lower

* Present address: Calcutta School of Tropical Medicine, Calcutta, India.

oesophagus and tied into the oesophagus at the neck excluding the vagus. The jugular veins were then exposed and cannulated with polythene tubes of 1 mm. diameter bevelled at the tip. The abdomen was opened through a midline incision, the pyloro-duodenal junction exposed and a small glass cannula introduced through a cut in the duodenum into the stomach and secured firmly by tying a ligature round the pylorus, care being taken not to include blood vessels within the ligature. The whole stomach was then brought forward and a longitudinal incision about 1¼ in. long was made with an electro-cautery knife along the middle of the anterior surface beginning as near the fundus as possible. Food particles were scooped out carefully with moist cotton-wool, special attention being paid to the fundal region and crevices between the mucous folds. The whole interior of the stomach was washed with cotton-wool soaked in warm saline and the mouth of the cannula was freed of food debris before closing up. The cut edges were then united and secured firmly by means of a continuous suture through the whole thickness of the wall so that no leakage of fluid occurred. Finally, the structures were returned to their proper places and the abdominal wound closed by two or three interrupted sutures with the free end of the cannula projecting. The whole operation lasted about 30 min.

Continuous Recording of Acid Secretion.—The stomach was perfused continuously with a dilute solution of NaOH, the fluid emerging from the pylorus passing over a glass electrode which recorded *p*H continuously. By the time the fluid had traversed the stomach it had collected sufficient buffer to act as an approximately linear buffer system over the *p*H range 6.5 to 4.5 when titrated with 0.1 N-HCl. There was thus an approximately linear relation between *p*H and acidity. The shape of the titration curve is presumably due to buffers of various kinds secreted by or diffusing from the mucosa of the stomach.

In most experiments N/4000-NaOH was perfused through the stomach at a rate of about 1 ml./min. When this solution was collected after having passed through the unstimulated stomach it gave an initial *p*H of the order of 6 to 6.5.

Gastric Perfusion System.—This is shown in Fig. 1. The solution of NaOH was kept in a Mariotte stock bottle which was fitted with a soda-lime tower and suspended above the preparation. The perfusion rate was controlled by a length of capillary resistance tubing connected to the stock bottle. This in turn was connected to a small jacketed warming coil (kept at 30° by a circulating pump) which was joined directly to the oesophageal tube. The capillary was chosen to give a flow of 1 ml./min. with a pressure of 200 cm., and the flow was maintained constant within 0.1 ml./min. The pyloric cannula was connected to a recording glass electrode through a short polythene tube. The glass electrode was placed 15 cm. below the level of the animal; a slight negative pressure was thus exerted, and prevented distension of the stomach.

Optimal conditions for an assay obtained when the perfusate emerging from the pylorus had an initial *p*H of about 6.5 which would decrease to about *p*H 4.0 by the action of stimulant drugs. An initial *p*H of 6.5 could generally be achieved by adjusting the perfusion rate within the limits of 1 to 2 ml./min. through variation in perfusion pressure, without changing the concentration of NaOH from the standard molarity of N/4000. In some experiments N/2000 or N/8000-NaOH was used.

The recording glass electrode was made of a lithium glass membrane; it had a diameter of 5 mm. and was fitted into a U-shaped glass container with a capillary lumen. One limb of the U-tube was slightly dilated to hold the glass electrode, and at this point the porous plug of the reference electrode was attached. The dead space within the glass container was 0.2 ml., and the total dead space from the gastric cannula to the bulb 1.3 ml. The whole assembly was held by clamps and a stand in such a way that the reference electrode was at a level 12 in. higher than the glass electrode. As a result of this pressure gradient, the KCl solution diffused slowly and steadily

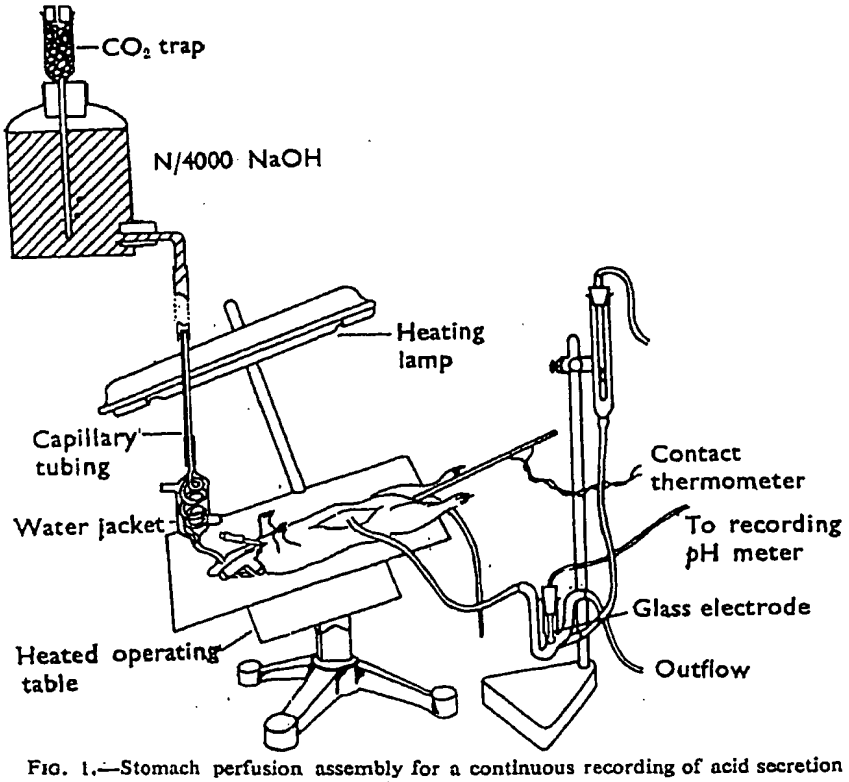


FIG. 1.—Stomach perfusion assembly for a continuous recording of acid secretion in the rat.

through the porous plug serving as an efficient salt-bridge connexion.

The electrodes were connected to a direct reading *p*H meter (Electronic Instruments Ltd., Model 23A) and thence to a circular ink recorder (Fielden's servograph recorder). There was no appreciable drift or lag in the apparatus and the stability of the recorder was within 0.1 *p*H unit. In the illustrations the record has been transformed to rectilinear co-ordinates. Drug responses have been expressed in terms of the maximum deflexion of the *p*H record from the base line.

All drugs were administered intravenously. The first injection was usually made 10 min. after completing the operation. Drugs were injected in a volume of 0.1 to 0.4 ml. followed by a washing injection of 0.1 ml. saline. The concentration of histamine acid phosphate is expressed in terms of the base, and that of the choline esters in terms of the hydrochlorides.

Results

The Effect of Histamine

A typical effect of histamine on acid secretion is shown in Fig. 2. Although the drug was administered by a rapid intravenous injection, it produced a delayed and gradually increasing effect. The *p*H began to fall 4 min. after the injection and reached the lowest point 13 min. later. Only a small fraction of this delay was attributable to mechanical lag in the perfusion system. A rough estimate of this lag could be obtained by injecting acid into the oesophageal tube ; when a dose of

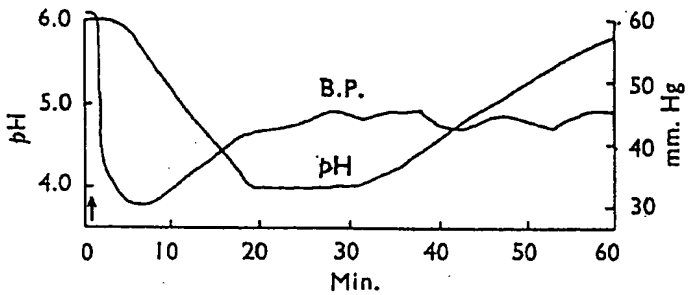


Fig. 2.—Effect of intravenous injection of 100 μg. histamine (at arrow) on blood pressure and *p*H of stomach perfusate. In this and the following illustrations rats anaesthetized with urethane and kept at 30° were used. Ordinates: *p*H of stomach perfusate on the left and blood pressure on the right.

0.1 ml. 0.1 N-HCl was so given, it produced a sharp fall of *p*H at the recording electrode within 40 sec., followed by 90% recovery in 2 min. A record of blood pressure taken simultaneously with the *p*H record in Fig. 2 shows that the two responses have a different time course. The blood pressure fell almost immediately after the intravenous injection and started recovering long before the secretory effect had reached its maximum.

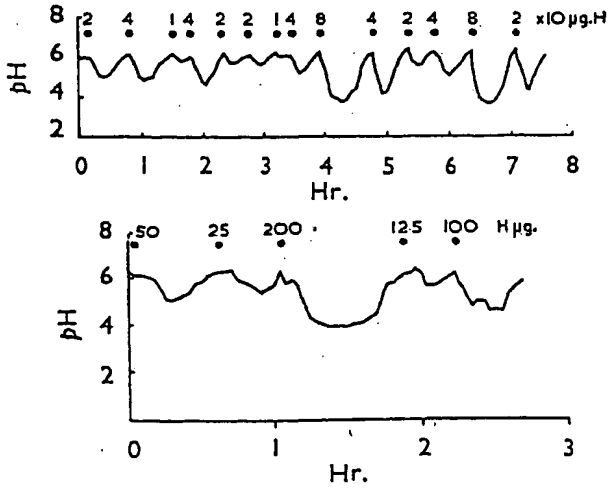


Fig. 3.—Stimulation of acid secretion by intravenous doses of histamine (H). Graded responses were obtained in one preparation with doses of 10 to 80 μg. and in the other with 12·5 to 200 μg. histamine.

Since the effect of an intravenous dose of histamine lasted only 40 to 60 min. several successive doses could be given in the course of an experiment lasting several hours. In the experiments illustrated in Fig. 3, doses over an 8-fold and 16-fold range given in random succession produced graded effects. Repeated administrations of the same dose usually produced constant effects as shown in Fig. 4.

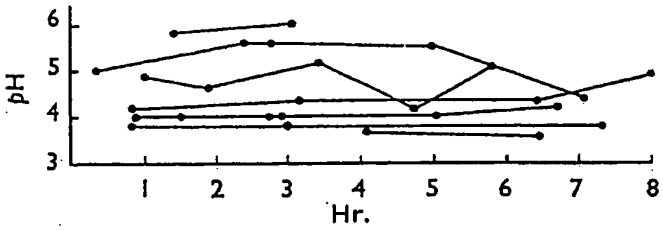


Fig. 4.—Effect of repeated administration of the same intravenous dose of histamine on acid secretion. Responses obtained in the same preparation are joined. The response to the first dose was omitted in each case.

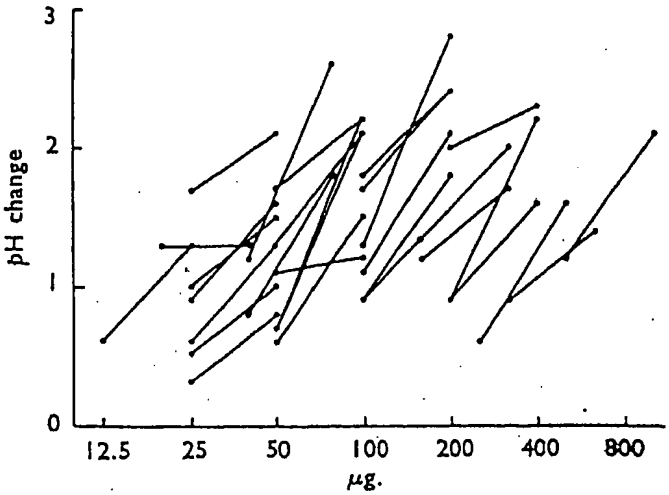


Fig. 5.—Effect of two doses of histamine on acid secretion in 30 rats.

There was a great deal of variation in the sensitivity of the rats. In some, 25 μg. histamine produced an average secretory response ; in others as much as 500 μg. was required, and about 10% failed to respond to doses of 1 mg. Fig. 5 shows the responses obtained with two graded doses of histamine in 30 rats. The average sensitivity of the preparations varied about 20-fold, but in each (except one) the effect increased with dose.

Effect of Body Weight.—Fig. 6 shows the histamine dose/100 g. of body weight required for a standard secretory effect when plotted against body weight. There is some evidence of a possible correlation between the two variables, suggesting that small rats are rather more sensitive to the secretory stimulus of histamine than large rats.

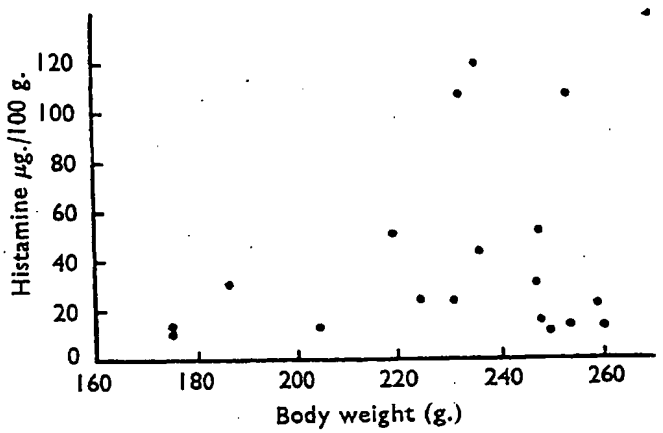


FIG. 6.—The dose of histamine/100 g. of body weight required to produce a standard secretory effect plotted against the body weight.

Effect of Body Temperature.—When the body temperature was changed by altering the external heat supply, the histamine response was consistently decreased by warming and increased by cooling. In one experiment, histamine produced a large effect at 30°, progressively smaller effects at 36°, and another large effect at 30°. In another experiment two large responses occurred at 30°, followed by a small response at 34°. Three further experiments are summarized in Fig. 7. In each, the magnitude of secretory response changed in a direction opposite to the body temperature, and this effect was reversible.

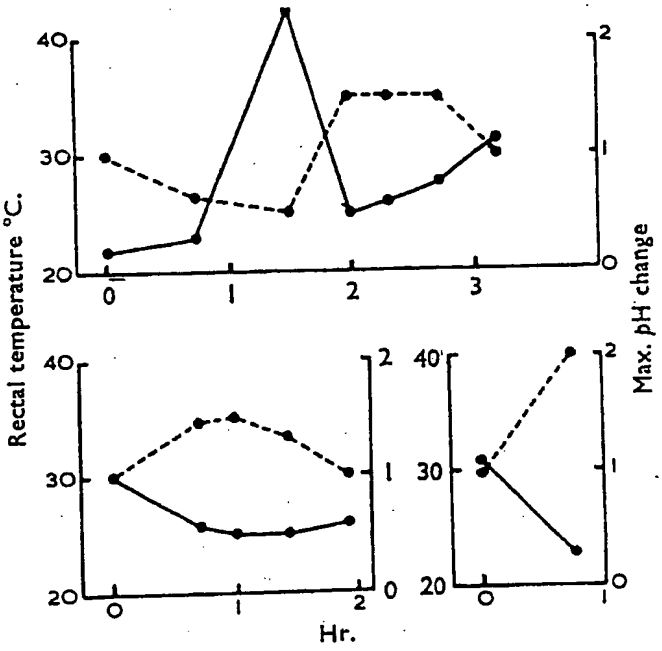


FIG. 7.—Effect of temperature on acid secretion induced by intravenous injections of a constant dose of histamine. Summary of three experiments. Temperature, broken line; *p*H, solid line.

Choline Esters

Acid secretion could be induced by intravenous injection of carbachol, methacholine, and acetylcholine. Acetylcholine was relatively inactive and tended to produce toxic side reactions culminating in respiratory arrest. It was nevertheless possible to record stimulation of secretion by acetylcholine several times. The effect of acetylcholine was potentiated by neostigmine. The degree of potentiation was related to the dose of neostigmine and the potentiation persisted for some time.

Methacholine was more active than acetylcholine. Graded responses were obtained within the range of 0.1 to 20 μg. The sensitivity to methacholine often increased in the course of an experiment. In one experiment the sensitivity increased nearly 100-fold in the course of 4 hours.

Carbachol proved a very powerful stimulant of secretion. In doses of 0.02 to 2 μg., it produced graded effects, the intensity and duration of which

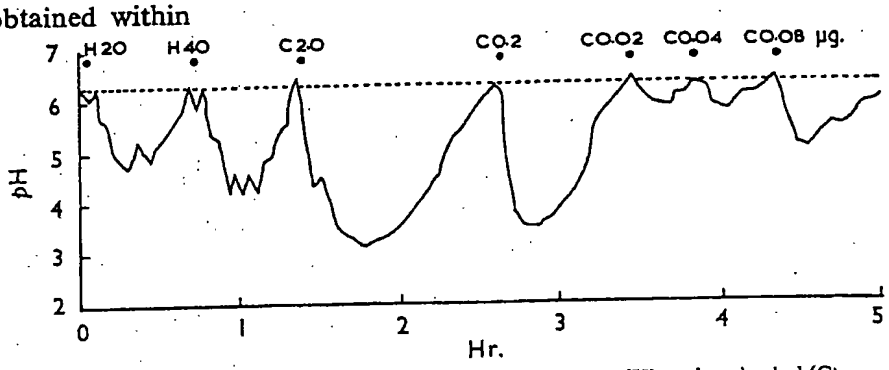


FIG. 8.—Secretory effects of graded doses of histamine (H) and carbachol (C).

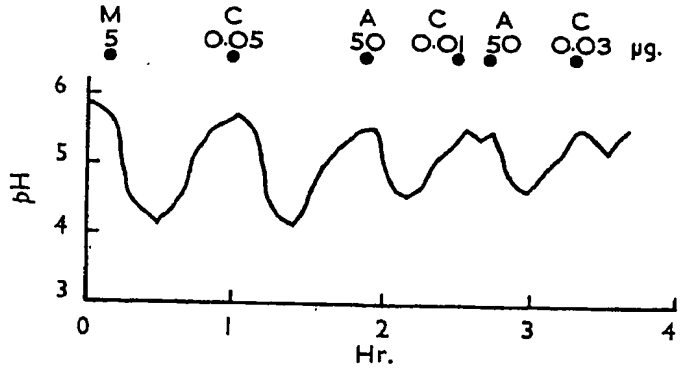


FIG. 9.—Comparison of secretory effects of methacholine (M), carbachol (C), and acetylcholine (A).

increased with the dose as shown in Fig. 8. Weight for weight, carbachol was considerably more active than histamine.

A direct comparison of the secretory effects of these three choline esters is shown in Fig. 9. In this experiment carbachol was 100 times as active as methacholine and about 1,000 times as active as acetylcholine. Table I summarizes the activity ratios obtained in six assays. Although the activity ratios varied in different preparations carbachol was obviously the most active, and acetylcholine the least active of the three compounds. The activity ratios for acetylcholine hold only for small

TABLE I

ACTIVITY RATIOS OF ACETYLCHOLINE, METHACHOLINE AND CARBACHOL

Drug Ratios	Exp. No.	Estimated Activity Ratios		
Methacholine / Acetylcholine	168	>10		<20
	179	>10		<40
	183	=200		>50
	191		>10	
			>25	
Carbachol / Acetylcholine	139		>1,000	
	168	>10		<100
	179		>500	
	183	>1,000		<1,600
	184	>250		=500

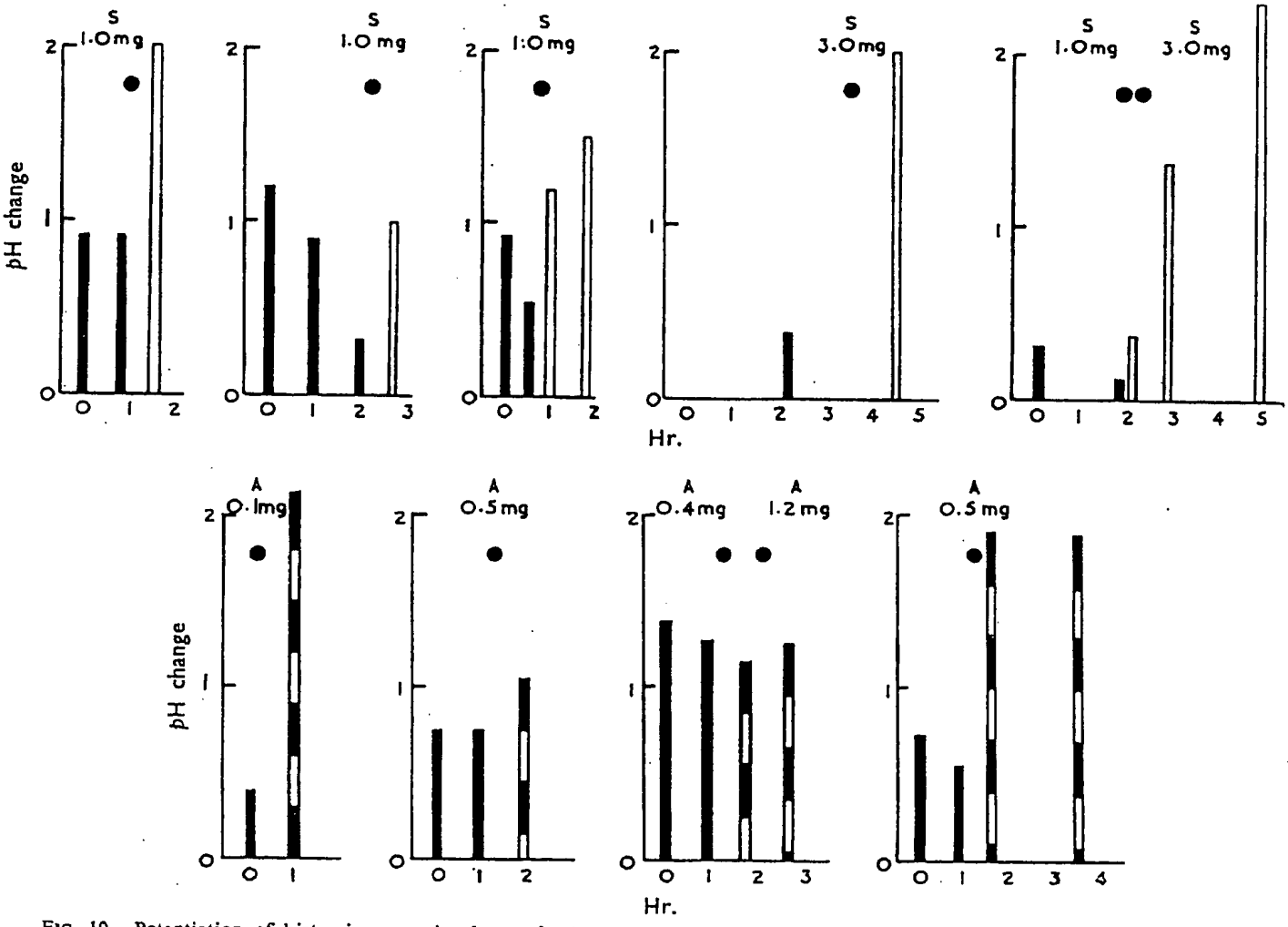


FIG. 10.—Potentiation of histamine secretion by semicarbazide (S) and aminoguanidine (A) in two different experiments. The dose of histamine in each experiment was kept constant.

or medium secretory effects, since doses of acetylcholine producing large effects were not administered owing to their toxicity.

The Effect of Antihistaminases

Two inhibitors of histaminase, aminoguanidine (Schuler, 1952) and semicarbazide (Zeller, 1942), were used. The antihistaminases were administered intravenously shortly before or together with the test drug, in doses in which they produced no secretion on their own.

Both aminoguanidine and semicarbazide produced a strong potentiation of histamine secretion. Semicarbazide was administered in five experiments in doses of 1 and 3 mg., some experiments involving several injections. The results are summarized diagrammatically in Fig. 10*a*, which shows that each time the effect of histamine was potentiated. Aminoguanidine was administered in five experiments in doses ranging from 0.1 to 1.2 mg. The results with aminoguanidine are summarized in Fig. 10*b*, which shows potentiation of histamine in all experiments except one.

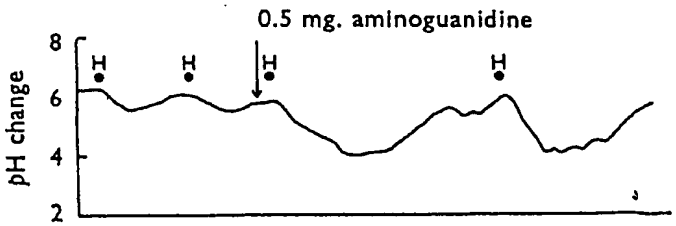


FIG. 11.—Persistence of potentiation of histamine (H = 400 μg.) secretion by aminoguanidine.

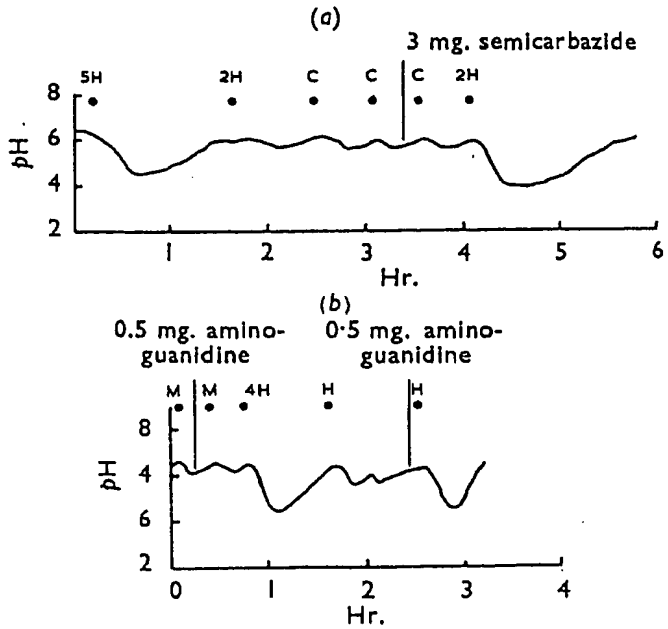


FIG. 12.—(*a*) The potentiation of histamine (H = 200 μg.) induced gastric secretion by semicarbazide but not by carbachol (C = 0·2 μg.) secretion. (*b*) Aminoguanidine potentiation of histamine secretion (H = 12·5 μg.) but not of methacholine (M = 50 μg.) secretion.

Potentiation by a single dose of antihistaminase sometimes persisted during subsequent injections of histamine; in one experiment a second dose of histamine given 1 hr. after the administration of aminoguanidine produced a greater effect than the first dose given with the aminoguanidine. An example of persistent potentiation is shown in Fig. 11. Two consecutive doses of 400 μg. histaamine produced only small effects, but the same dose of histamine was still potentiated when given 2 hr. after 500 μg. of aminoguanidine. Neither methacholine- nor carbachol-induced secretion was appreciably increased by antihistaminases. Fig. 12 shows experiments in which antihistaminases were tested with histamine and with choline esters in the same preparation. Fig. 12*a* shows no potentiation of carbachol by semicarbazide, but a strong potentiation of histamine at a later stage. Fig. 12*b* shows no potentiation of methacholine by aminoguanidine, but potentiation of histamine. In two other experiments, antihistaminases produced a slight potentiation of the effects of choline esters; but this was variable and not comparable in intensity with the potentiation of histamine.

Discussion

The method of continuous recording of *p*H has obvious limitations. It measures total acid secretion, but gives no indication of the volume and acidity of the gastric juice, and the artificial conditions under which the animal is maintained, namely the low body temperature, anaesthesia and alkalinity of the stomach perfusate, may affect both acid and mucus secretion. On the other hand, the method has certain advantages for bioassay work; it is sensitive and follows the time course of secretion closely, so that successive doses of drugs can be administered as soon as the effect of a previous dose subsides.

In the early stages of this work distilled water or saline was used to perfuse the stomach; but it soon became apparent that these fluids were unsuitable since they gave initial *p*H values of 4 to 5 which were little affected by injections of histamine. Perfusion of the stomach with a linear buffer also proved unsuitable. A linear buffer has the theoretical advantage of a more truly linear relationship between acidity and *p*H, but the concentration-action curve becomes flat.

The procedure for recording *p*H continuously, which may be applicable to other species, seemed to depend for its success on the following points: (1) Low body temperature. Anaesthetized rats survived better at 30° than at 37° or even at

34°, and both the circulation and respiration were less liable to fail. (2) Cleaning of stomach. The difficulties of clearing the rat stomach of food residues by fasting are well known. We found that after 24 hr. fasting only about 80 to 90% of animals were free of food residue and that even longer fasting periods were frequently unsuccessful. When the unopened stomachs of these animals were perfused through the oesophagus it was not possible to clear the fundus completely of food residue and the secretory activity appeared very irregular. Opening of the stomach by a wide incision over the major curvature, and scooping out the contents, obviated these difficulties and produced surprisingly little interference with gastric secretion. There was no leakage from the operation wound, and the animals were not devitalized by fasting. (In some recent experiments the unopened stomach was washed out through a thin catheter introduced by way of the pylorus cannula. Satisfactory recordings were obtained by this method.) (3) Urethane anaesthesia. Since anaesthetics affect gastric secretion (Schachter, 1949), it is essential in any bioassay to keep the depth of anaesthesia constant. This cannot be achieved by anaesthetics such as pentobarbitone, which have a short duration of action and must therefore be administered repeatedly. Urethane when given in a single dose produced an apparently unchanged anaesthesia for at least 8 hr.

The Effect of Histamine

When an intravenous dose of histamine is given to rats the blood-pressure response is immediate, but the secretory response has a characteristic latent period and reaches a maximum only after about 15 min. This delay, which is surprising since the plasma concentration of histamine must be at its peak immediately after the injection, could be explained in two ways. It might be due to the slow rate at which secreted acid reaches the surface of the stomach. A second dose of histamine would then be expected to produce less delay since the necks of the glands would still be filled with secretion, but, in fact, the response to a second dose is equally delayed. Alternatively, the delay may indicate that the oxyntic cell responds only to a protracted stimulus. It is known that gastric secretion cannot readily be induced in the dog by single intravenous injections of histamine (Popielski, 1920 ; Rothlin and Gundlach, 1921 ; Ivy and Javois, 1925 ; but see Schofield, 1957), but only by slow infusions, and it may be that in rats a single intravenous dose of histamine circulates for some time in the blood stream and acts like a slow infusion. Single intravenous doses of histamine may produce a better gastric secretory response in rats simply because of their high tolerance for histamine, so that it is possible to administer large doses which take a long time to disappear from the blood stream.

The Effect of Antihistaminases

Antihistaminases produce a specific potentiation of histamine secretion in the rat. This agrees with their specific potentiating effect in other species and preparations (Arunlakshana, Mongar and Schild, 1954 ; Westling, 1956) but contrasts with their effect on gastric secretion in dogs. Sircus (1953) and Ivy, Lin, Ivy, and Karvinen (1956) found in dogs that antihistaminases potentiate not only histamine secretion but also secretion induced by carbachol, insulin and feeding. These results were interpreted in terms of a release and subsequent potentiation of histamine from the gastric mucosa by these various stimuli. This would support the theory of histamine as the final common path for gastric secretion (MacIntosh, 1938 ; Babkin, 1938) ; but against this interpretation is the fact that the gastric mucosa contains no histaminase to account for the potentiation. In rats, however, the antihistaminases potentiate histamine secretion selectively, and it is thus unnecessary to postulate that they act on the gastric mucosa ; they presumably inhibit histaminase elsewhere in the body, for example in the kidney and intestine (Waton, 1956), and, in this way, prolong the circulation time of injected histamine.

Direct Assays

Direct assays of secretory stimulants can be performed in this preparation by injecting the drugs intravenously and matching their effects. Carbachol was shown to be 50 times as active as methacholine and 1,000 times as active as acetylcholine. When the choline esters were injected intravenously they exhibited the same sort of latent period and gradually increasing secretory effects as histamine ; it would thus seem that the delay in response observed with histamine is not a characteristic of the drug but of the secretory cell. The high secretory activity of carbachol agrees with results obtained in other species ; in the rat it is presumably due, partly at least, to stimulation of ganglia since it was found (unpublished observations) to be reduced by hexamethonium. The low secretory activity of acetylcholine in the rat also agrees with results in other species (Necheles, Mortel, Kosse and Neuwelt.

1938 ; Uvnas, 1948 ; Morton and Stavraky, 1949 ; Pevsner and Grossman, 1955) and can be explained by a combination of two factors, firstly the slowness of the response of the oxyntic cell, and secondly the action of cholinesterase. In the presence of neostigmine the secretory effects of acetylcholine were indeed strongly potentiated.

This work was aided by a grant from the Medical Research Council.

REFERENCES

Arunlakshana, O., Mongar, J. L., and Schild, H. O. (1954). *J. Physiol.*, **123**, 32.

Babkin, B. P. (1938). *Canad. med. Ass. J.*, **38**, 421.

Flexner, J., and Kniazuk, M. (1940). *Amer. J. dig. Dis.*, 7, 138.

Ghosh, M. N., and Schild, H. O. (1955). *J. Physiol.*, **128**, 35P.

Ivy, A. C., and Javois, A. J. (1925). *Amer. J. Physiol.*, **71**, 604.

—— Lin, T. M., Ivy, E. K., and Karvinen, E. (1956). Ibid., **186**, 239.

Komarov, S. A., Shay, H., Rayport, M., and Fels, S. S. (1944). *Gastroenterology*, 3, 406.

MacIntosh, F. C. (1938). *Quart. J. exp. Physiol.*, **28**, 87.

Morton, G. M., and Stavraky, G. W. (1949). *Gastroenterology*, **12**, 808.

Necheles, H., Mortel, W. G., Kosse, J., and Neuwelt, F. (1938). *Amer. J. dig. Dis.*, **5**, 224.

Pevsner, L., and Grossman, M. I. (1955). *Gastroenterology*, **28**, 493.

Popielski, L. (1920). *Pflüg. Arch. ges. Physiol.*, **178**, 214.

Roe, J. H., and Dyer, H. M. (1939). *Proc. Soc. exp. Biol., N.Y.*, **41**, 603.

Rothlin, E., and Gundlach, R. (1921). *Arch. int. Physiol.*, **17**, 59.

Rovelstad, R. A., Owen, C. A., Jr., and Magath, T. B. (1952). *Gastroenterology*, **20**, 609.

Schachter, M. (1949). *Amer. J. Physiol.*, **156**, 248.

Schofield, B. (1957). *J. Physiol.*, **137**, 31P.

Schuler, W. (1952). *Experientia*, **8**, 230.

Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., and Siplet, H. (1945). *Gastroenterology*, **5**, 43.

Sircus, W. (1953). *Quart. J. exp. Physiol.*, **38**, 25.

Uvnas, B. (1948). *Acta physiol. scand.*, **15**, 1.

Waton, N. G. (1956). *Brit. J. Pharmacol.*, **11**, 119.

Westling, H. (1956). *Acta physiol. scand.*, **38**, 91.

Zeller, E. A. (1942). *Advanc. Enzymol.*, **2**, 93.